Genomics, models and therapeutic targets of genetic skeletal disorders

Carlos R. Ferreira, MD
Unit on Skeletal Genomics Unit

NICHD Advisory Council

September 04, 2024

Outline

- Background
- Disorders of skeletal development of unknown cause
  - Trevor disease
- Disorders of FGF23/phosphate axis (biomineralization)
  - ENPP1 deficiency

Unit on Skeletal Genomics – Mission

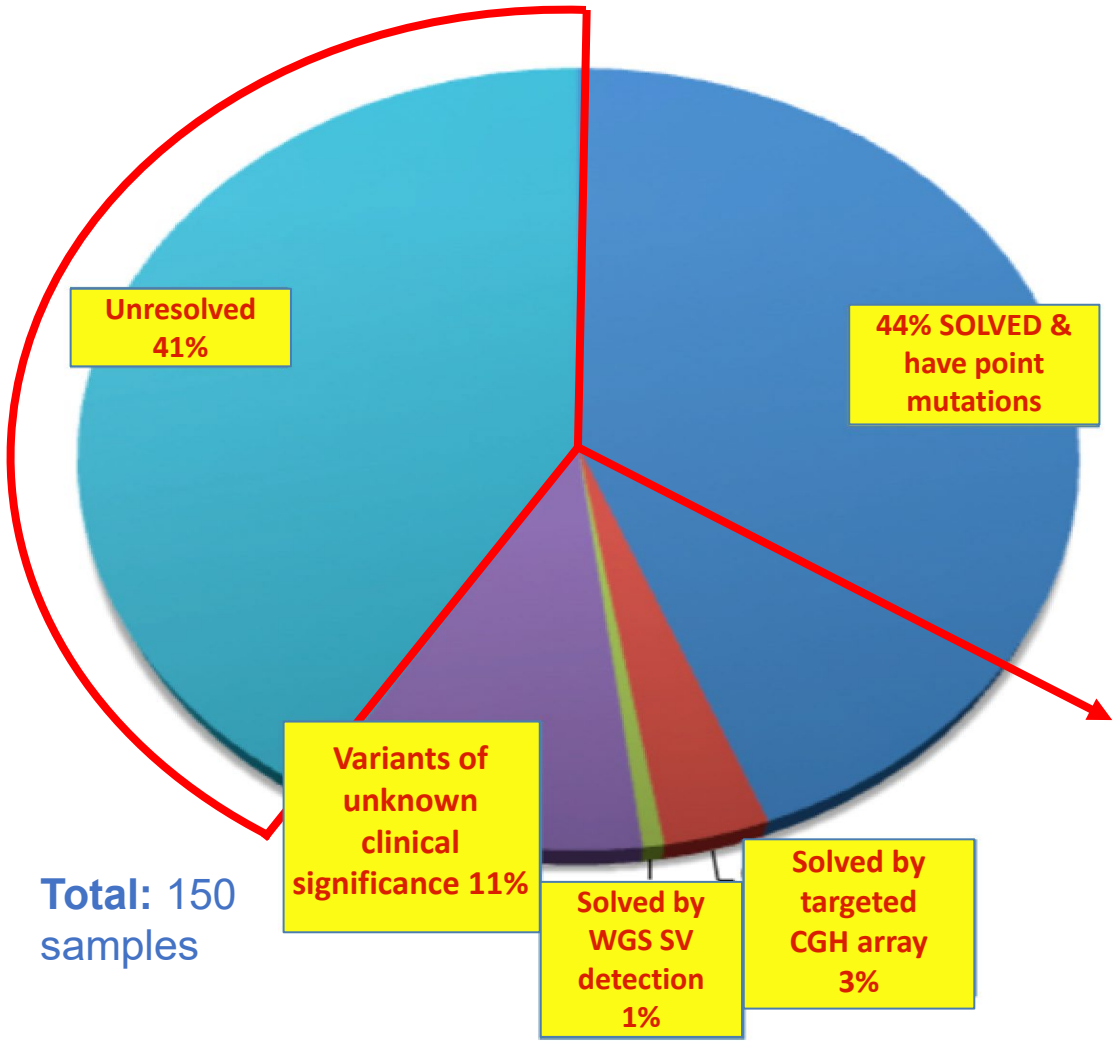
- Conduct natural history study to uncover the clinical spectrum of selected skeletal dysplasias and identify new gene-disease associations
- Understand their pathomechanisms via cell and animal models
- Develop targeted treatment approaches

Dedicated natural history study

- “Clinical and Laboratory Study of Rare Skeletal Disorders” (000213-HG; ClinicalTrials.gov Identifier: NCT05031507)], approved in 2021

- Focus on
  - disorders affecting skeletal development with unknown molecular basis
  - disorders of FGF23/phosphate axis

Disorders with unknown etiology



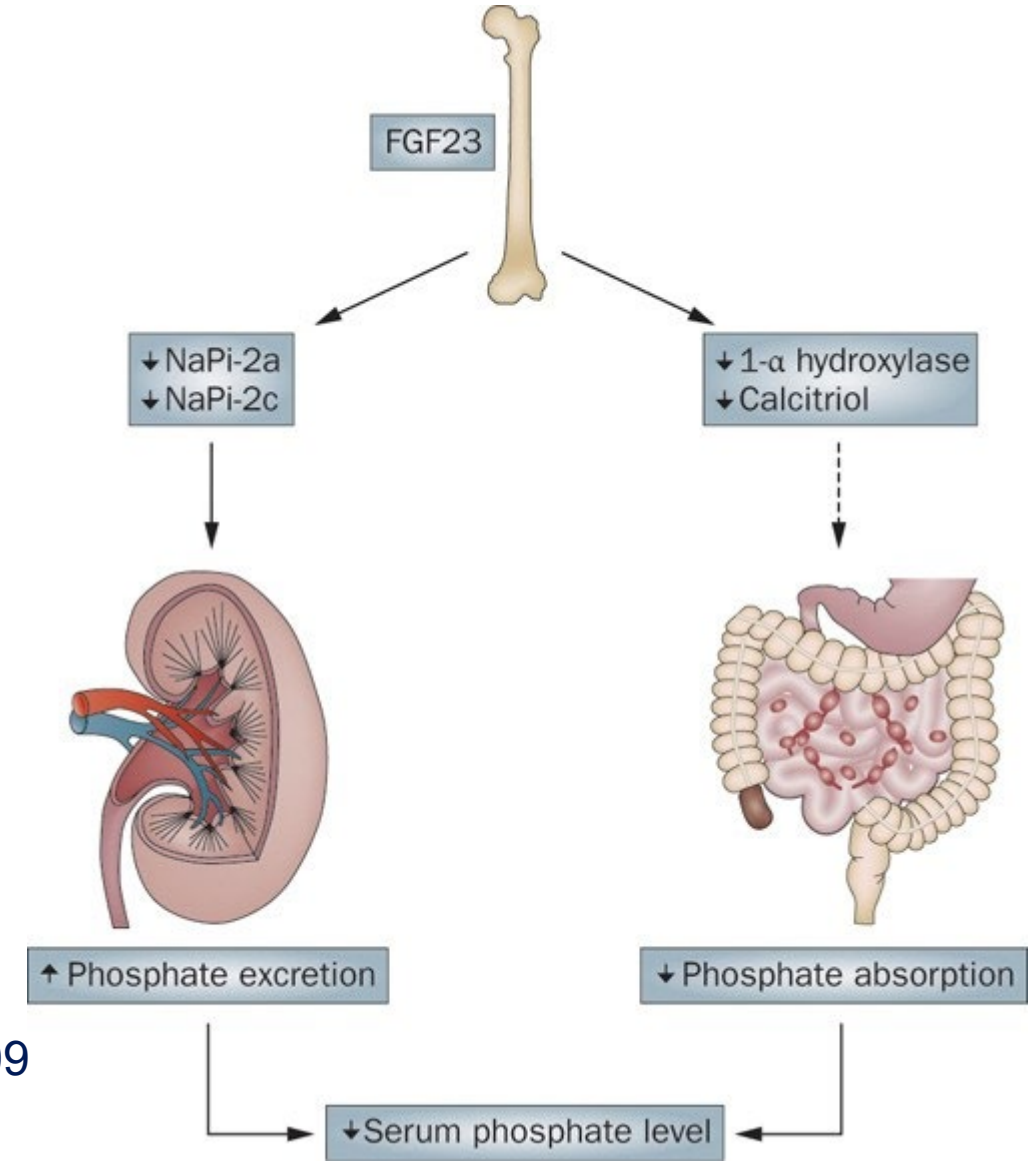
Courtesy of Prof. Grigelioniene (Karolinska Institute)

- Nosology (2023): 34 known disorders with unknown etiology

  Unger, Ferreira et al. *American Journal of Medical Genetics part A*, 2023

- Majority of new gene-disease associations not included in the prior nosology (2019)
- ~40% patients in large cohorts with no confirmed diagnosis
- Possibility of understanding new aspects of skeletal biology

FGF23-phosphate axis

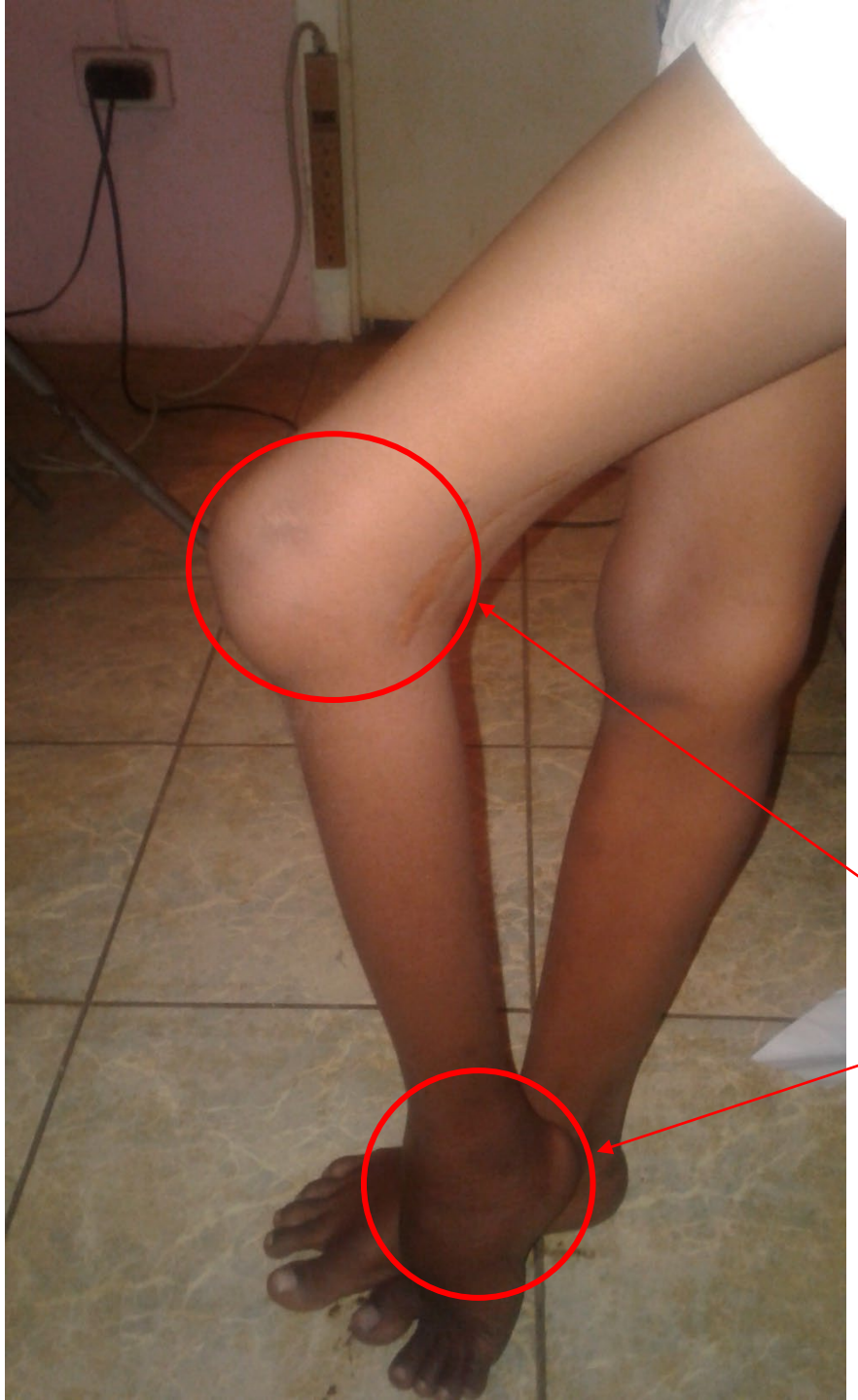


Razzaque. *Nature Reviews Endocrinology;* 2009

Trevor Disease, a mosaic skeletal disorder

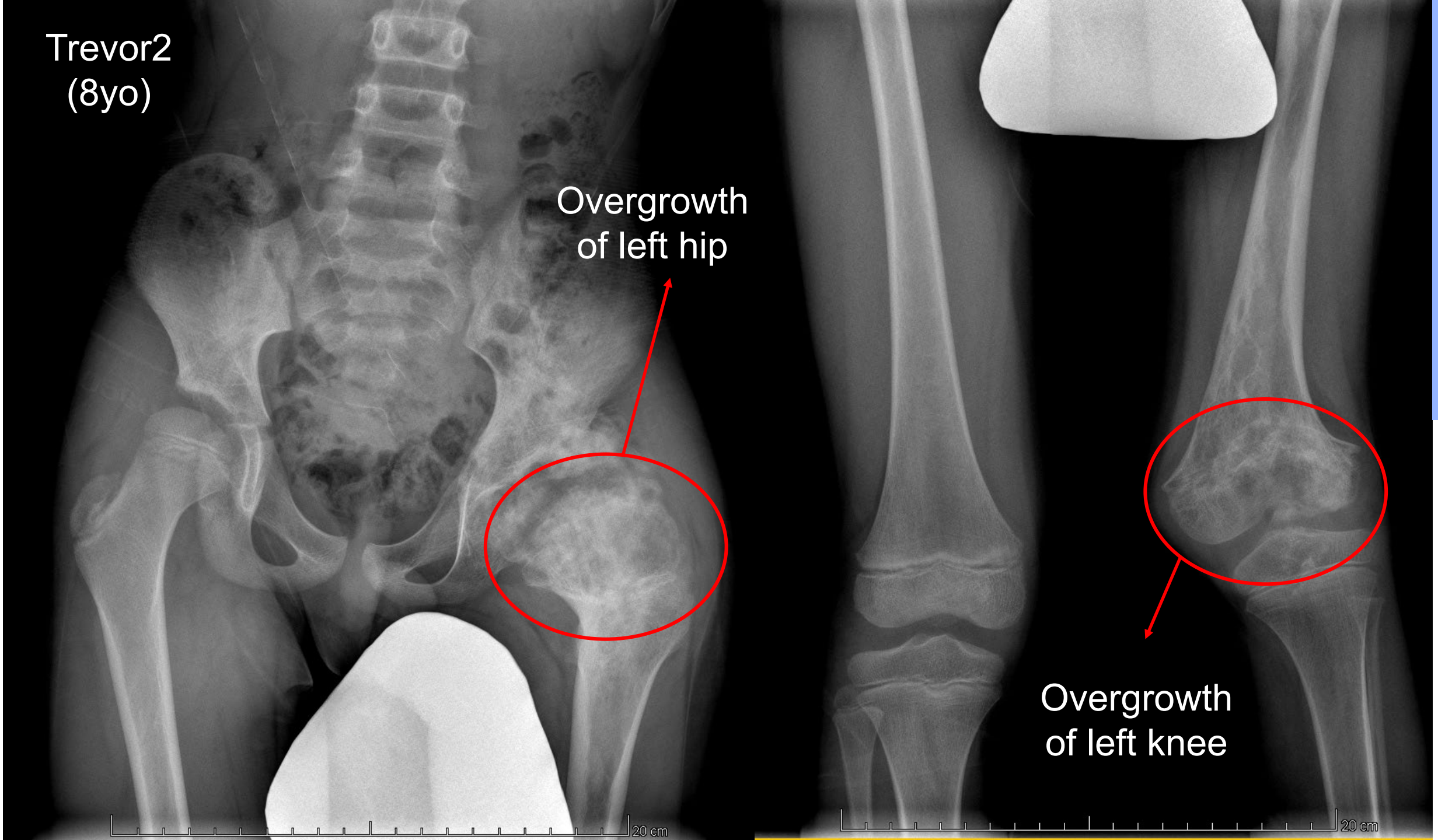
Trevor Disease (dysplasia epiphysealis hemimelica)

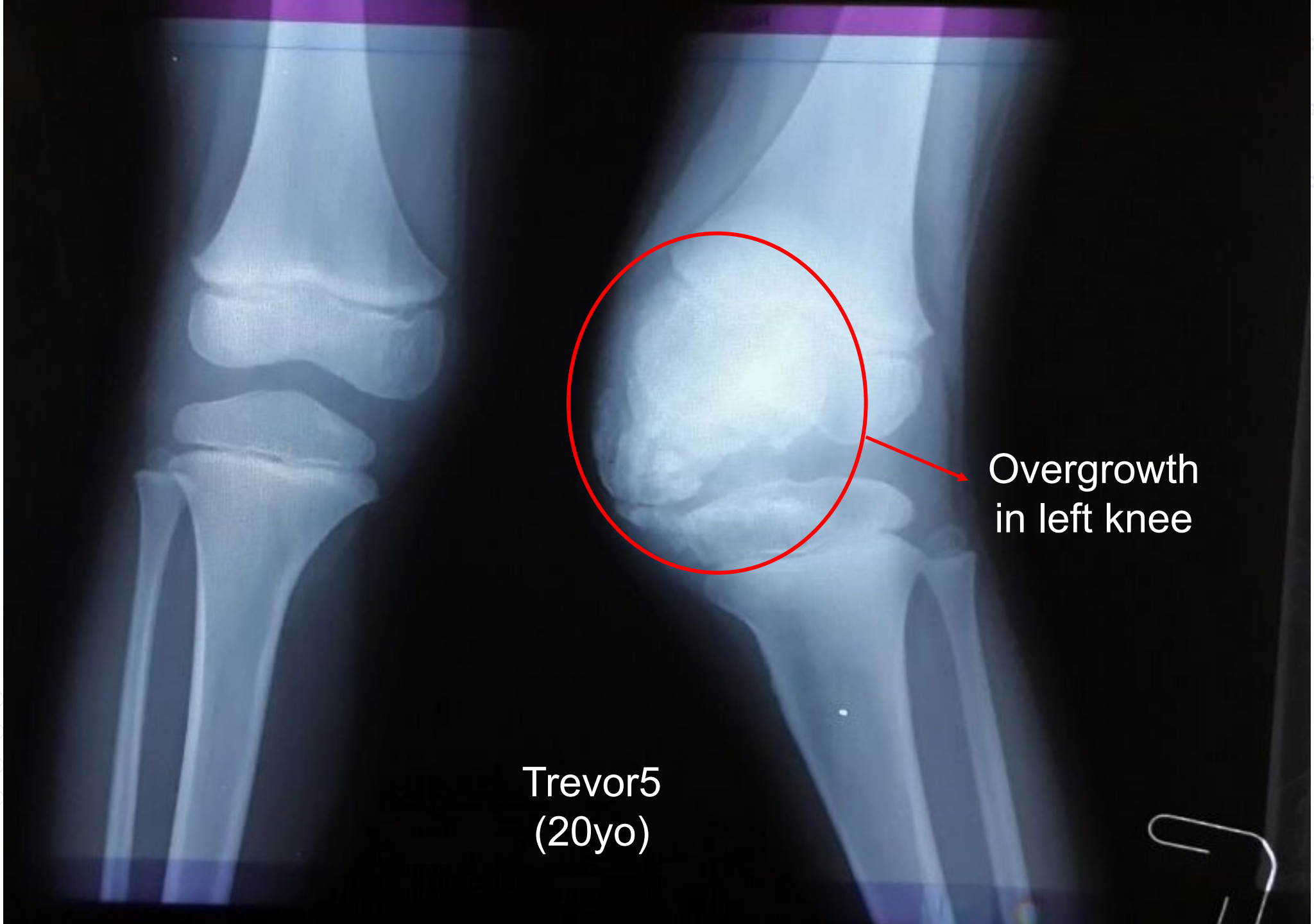
- Epiphyseal overgrowth
  - Histology: osteocartilaginous tissue



Joint contractures

Trevor1


Trevor2
(8yo)
Overgrowth
of left hip
Overgrowth
of left knee
20 cm
20 cm

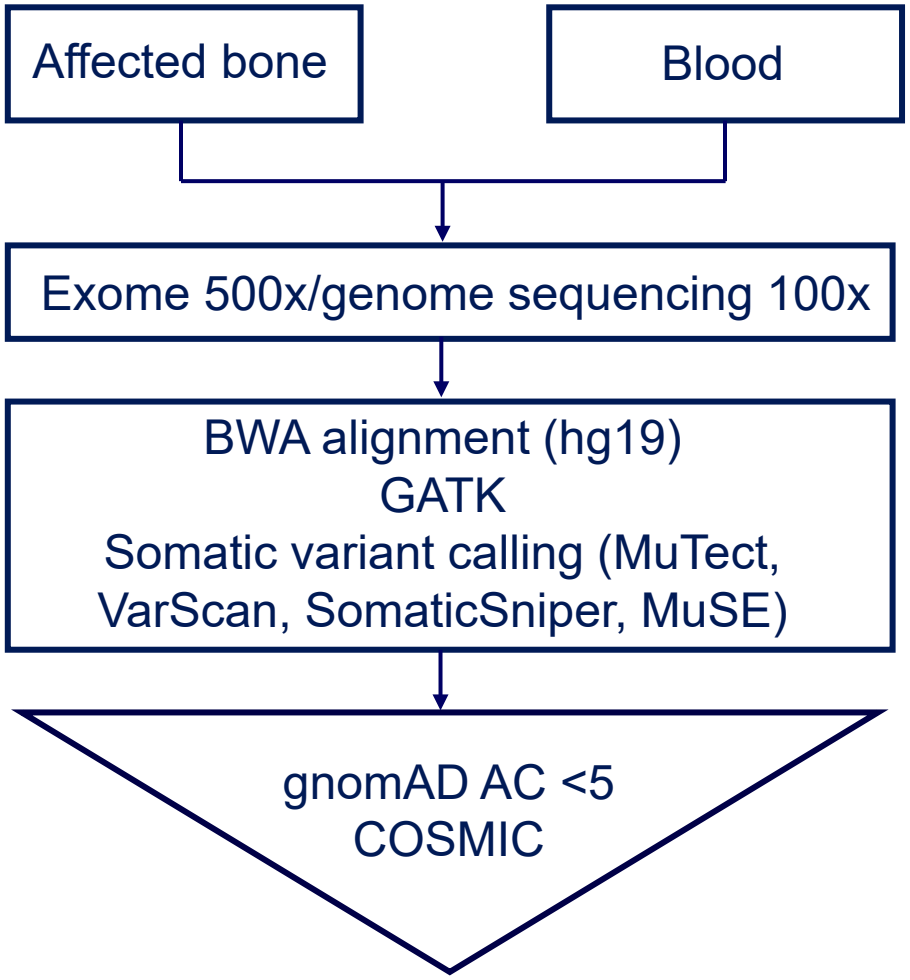

Overgrowth
in left knee
Trevor5
(20yo)

Mosaic disorder

- Lesions typically unilateral/localized
- No familial case
- Reported patient whose monozygotic twin was not affected

Deep sequencing to identify somatic variant

Bone biopsies from 3 subjects with Trevor disease

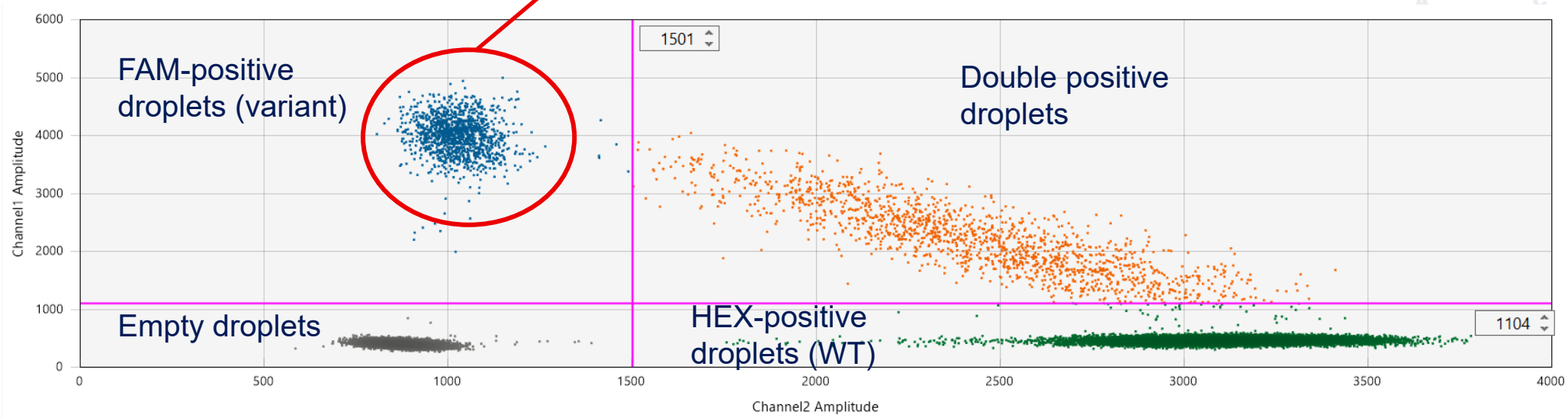


Quantitation of rare candidate variant

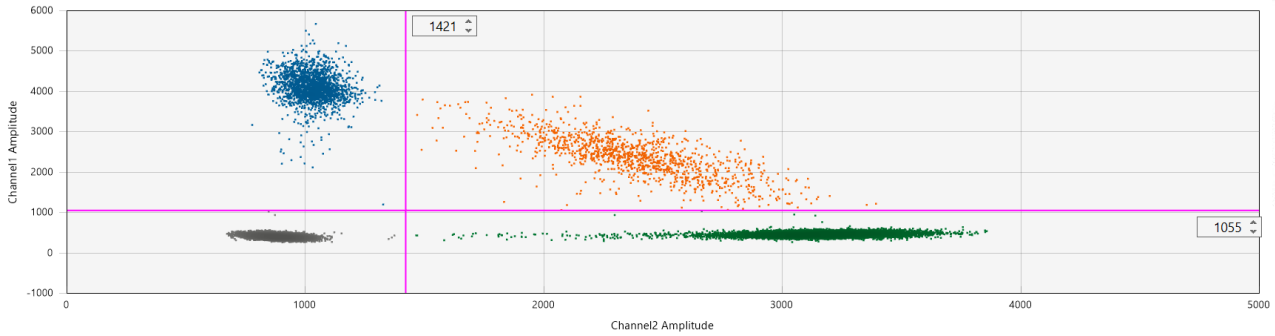
Preliminary data

Variant allele

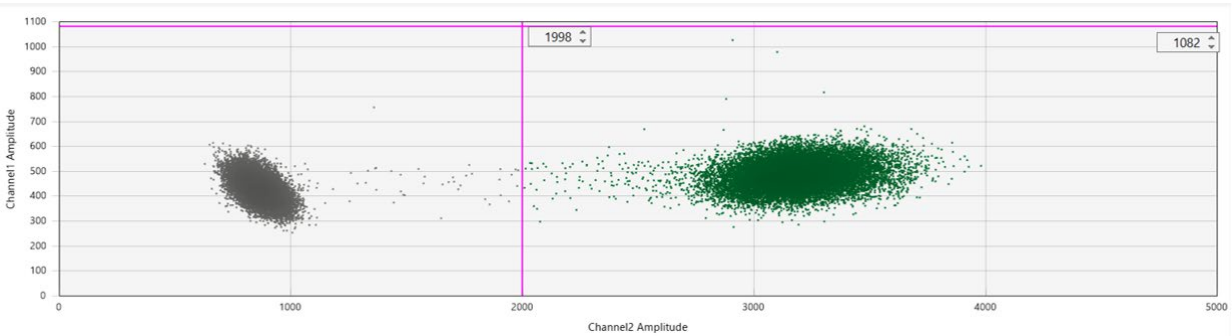
Trevor2_Bone (VAF 9.4%)



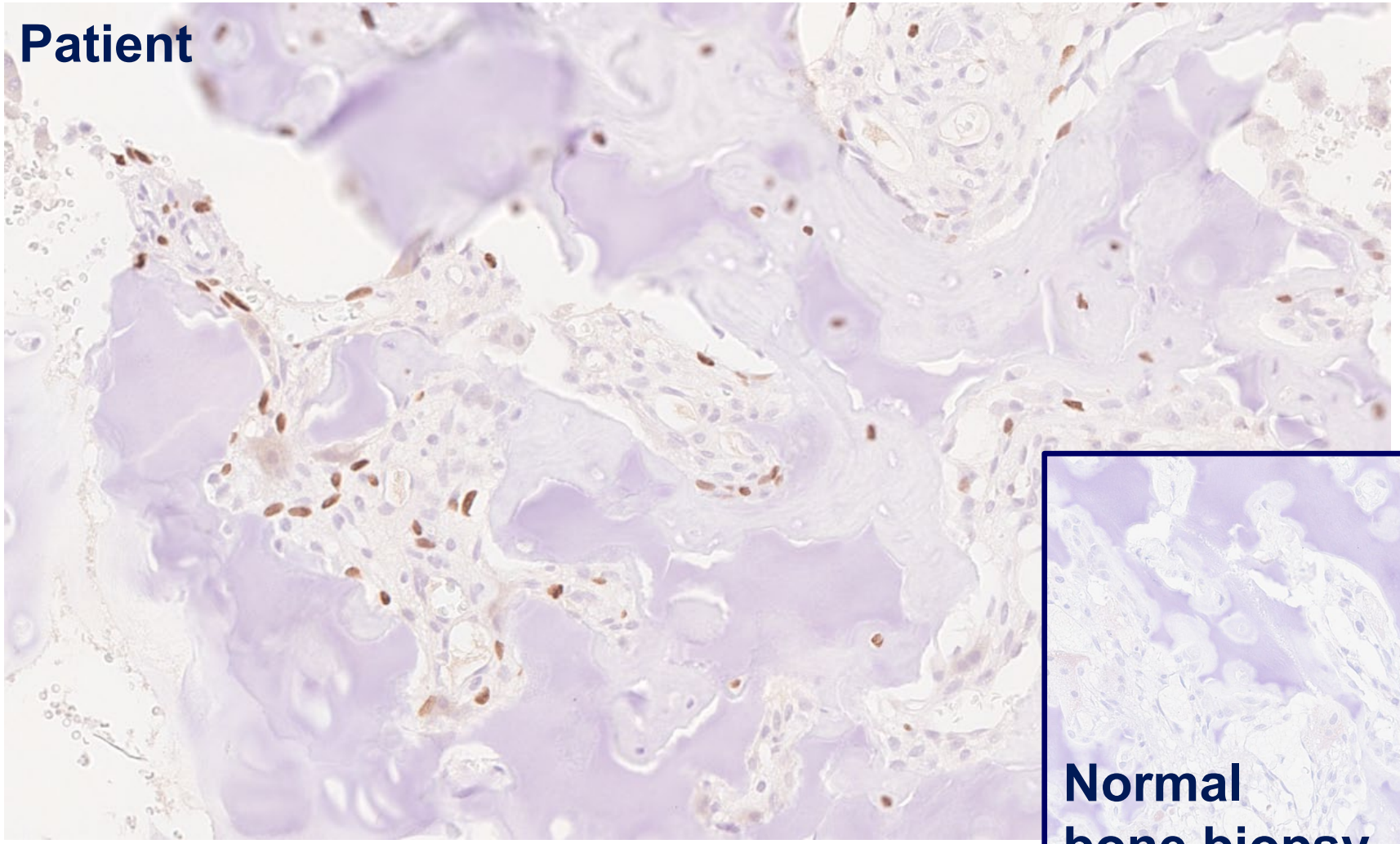
Trevor2_BMSCs (VAF 16.7%)

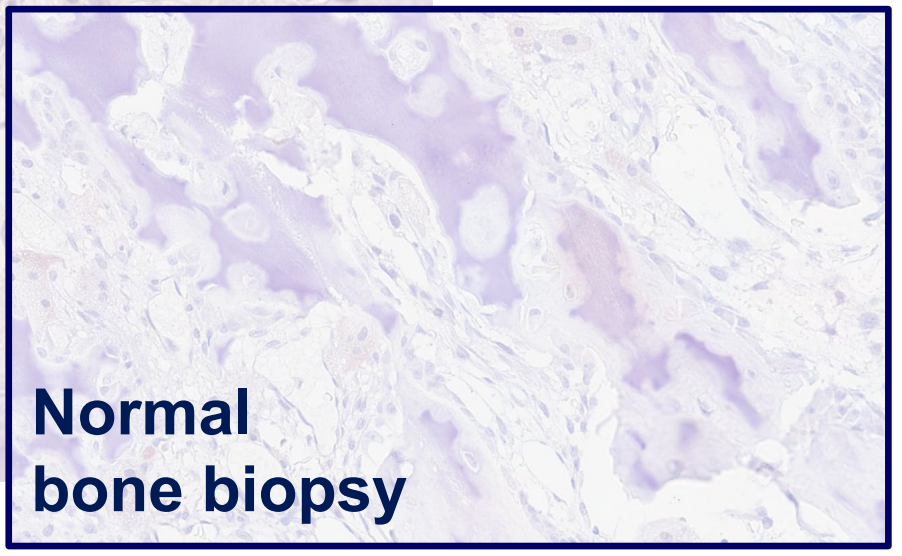


Trevor2_Blood (VAF 0%)



IHC to detect mutant protein in patient bone biopsy



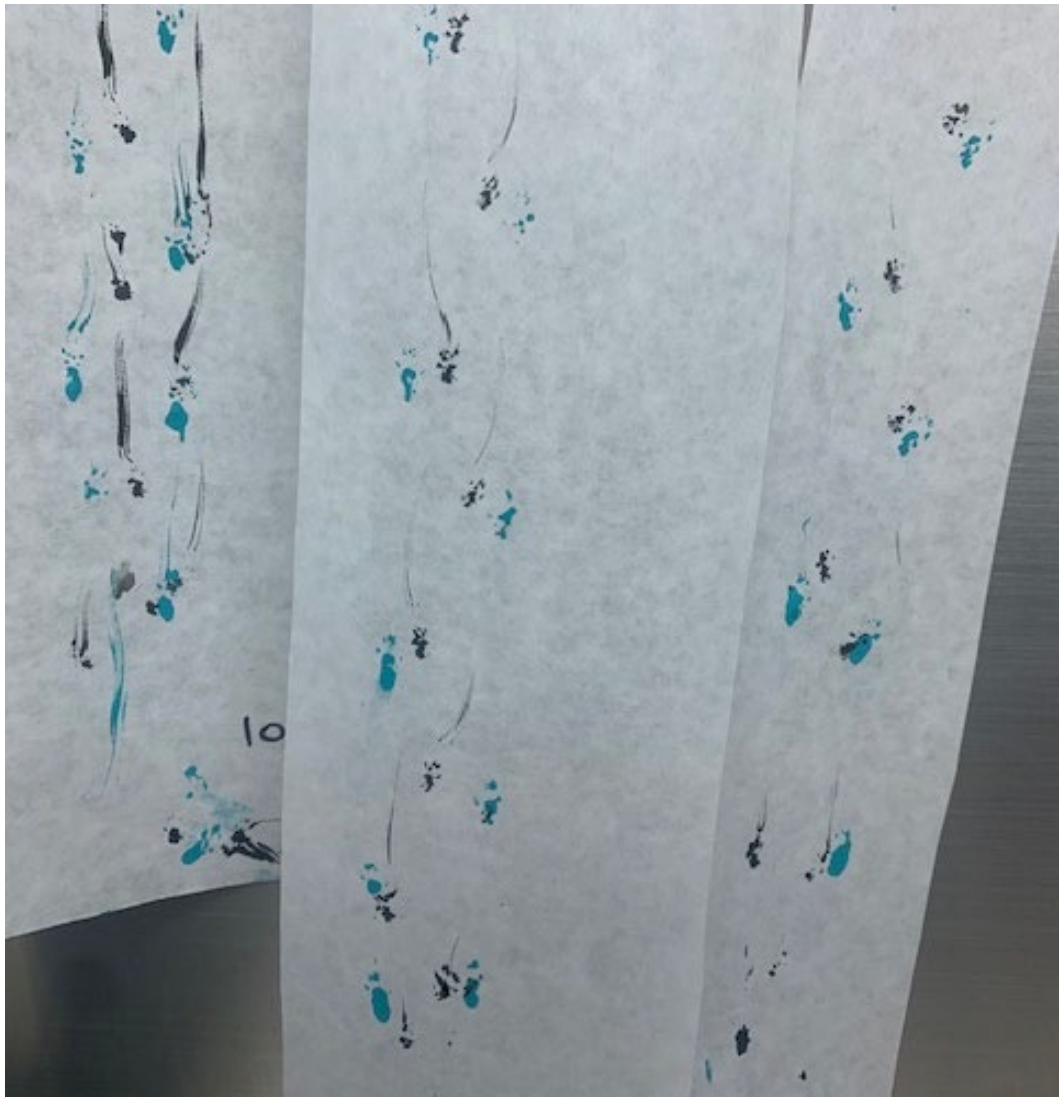


Preliminary data

Abnormal ambulation in the mouse model of Trevor disease

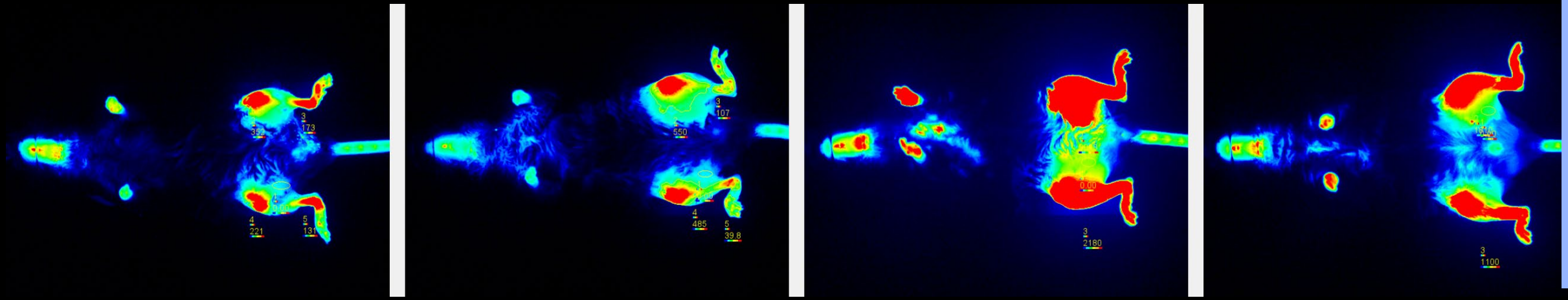
Control mice

Mutant mice



Preliminary data

Mutant mice have hyperostosis (optical imaging)

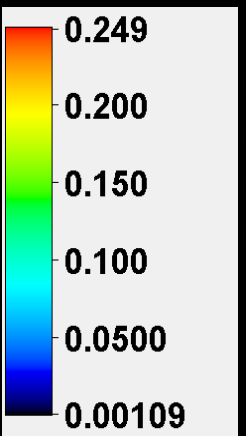


Control, M 22w

Control, M 23w

Mutant, F 22w

Mutant, M 23w

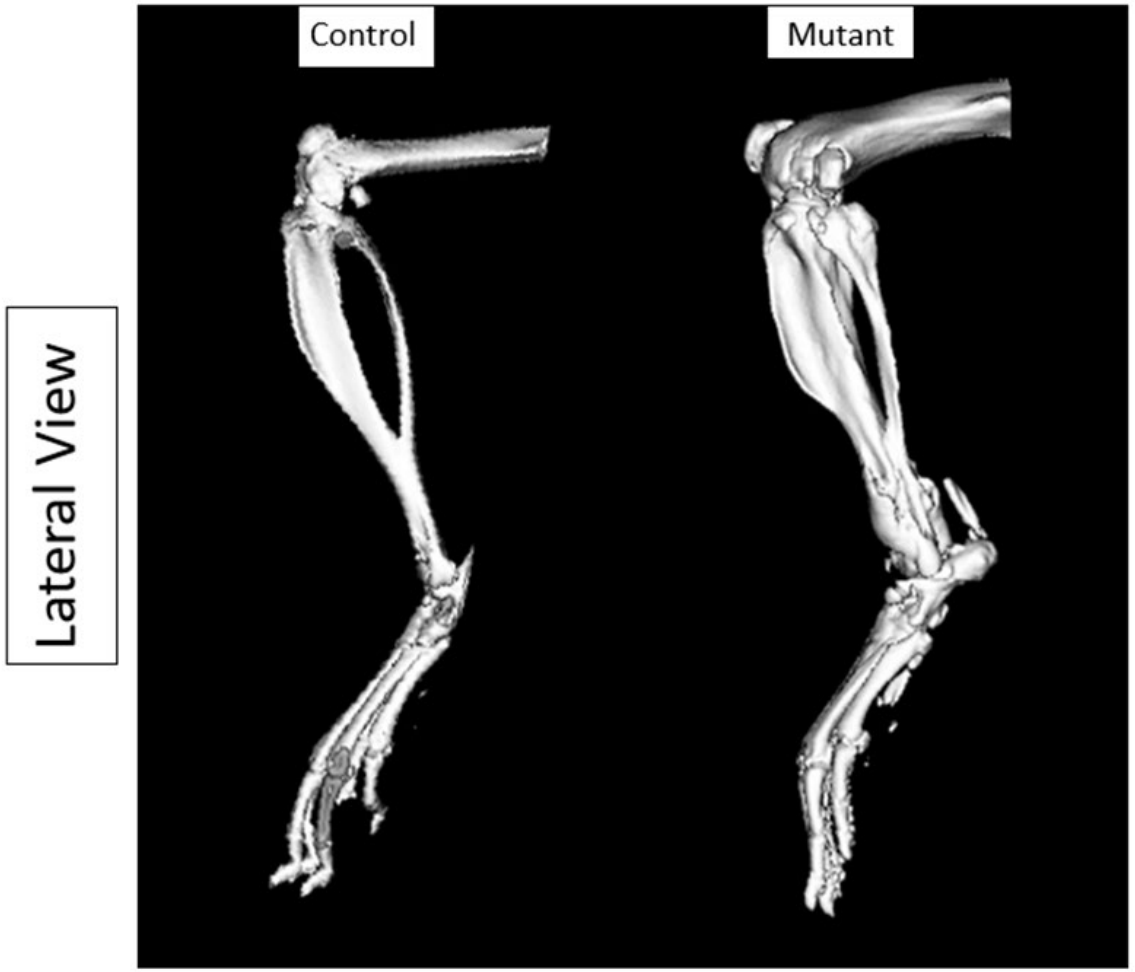


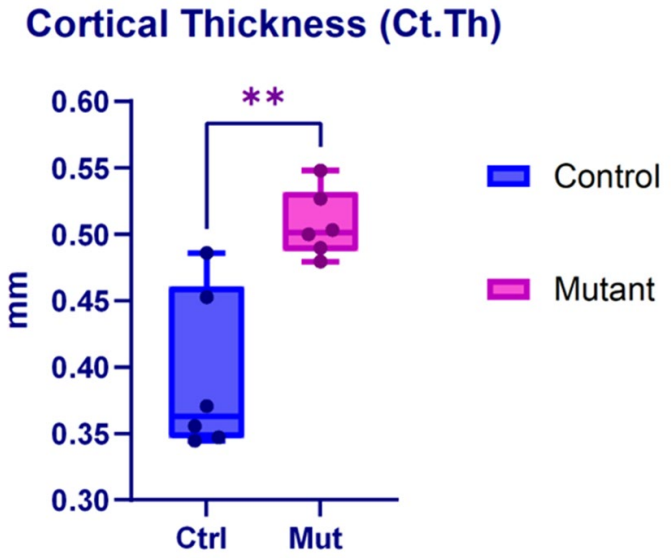
24 hours post injection of IRDye® 800CW BoneTag™ Optical Probe

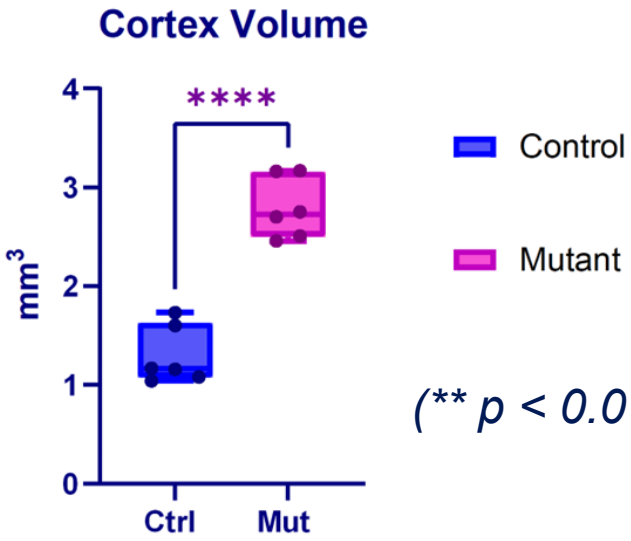
Preliminary data

Mutant mice have cortical hyperostosis

Preliminary data

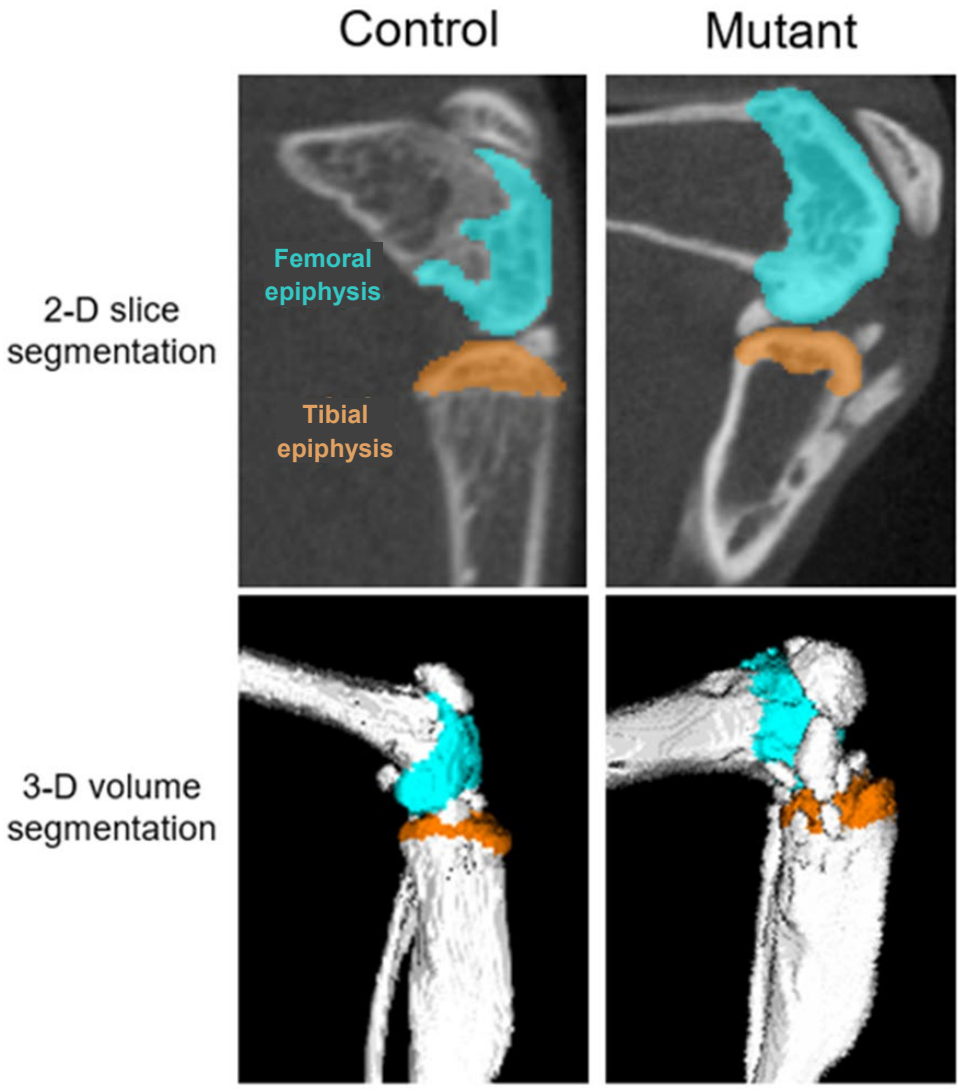


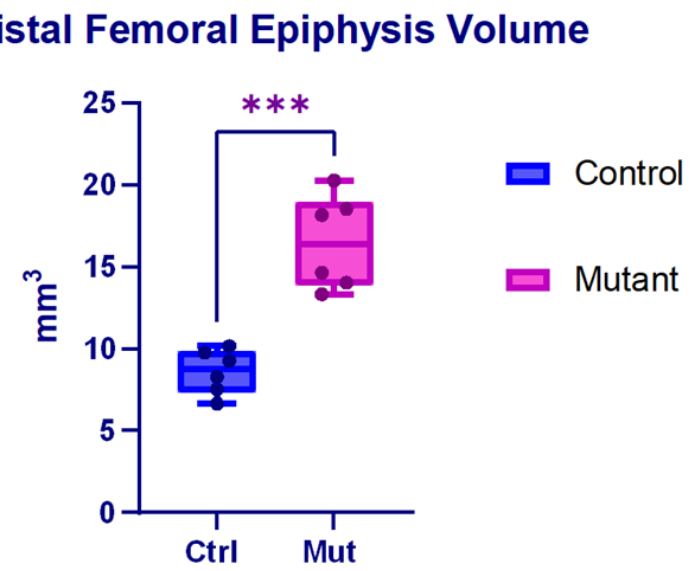


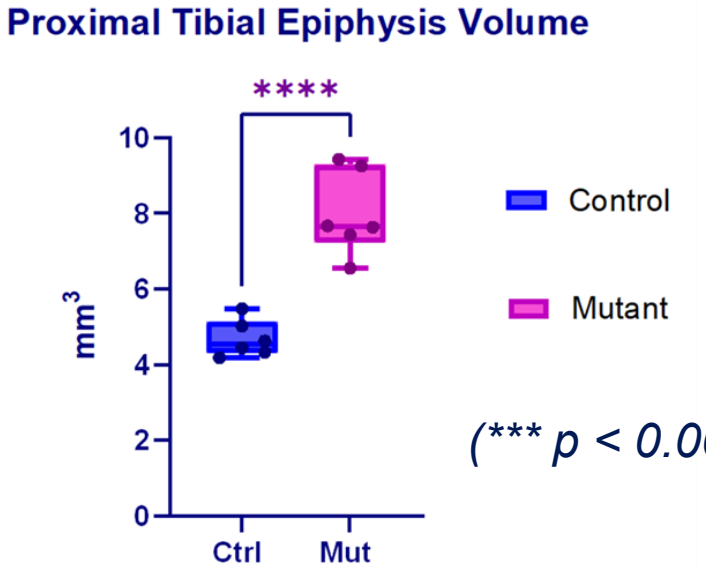


*(** p < 0.01; ****p < 0.0001)*

Mutant mice have increased epiphyseal volumes as seen in patients







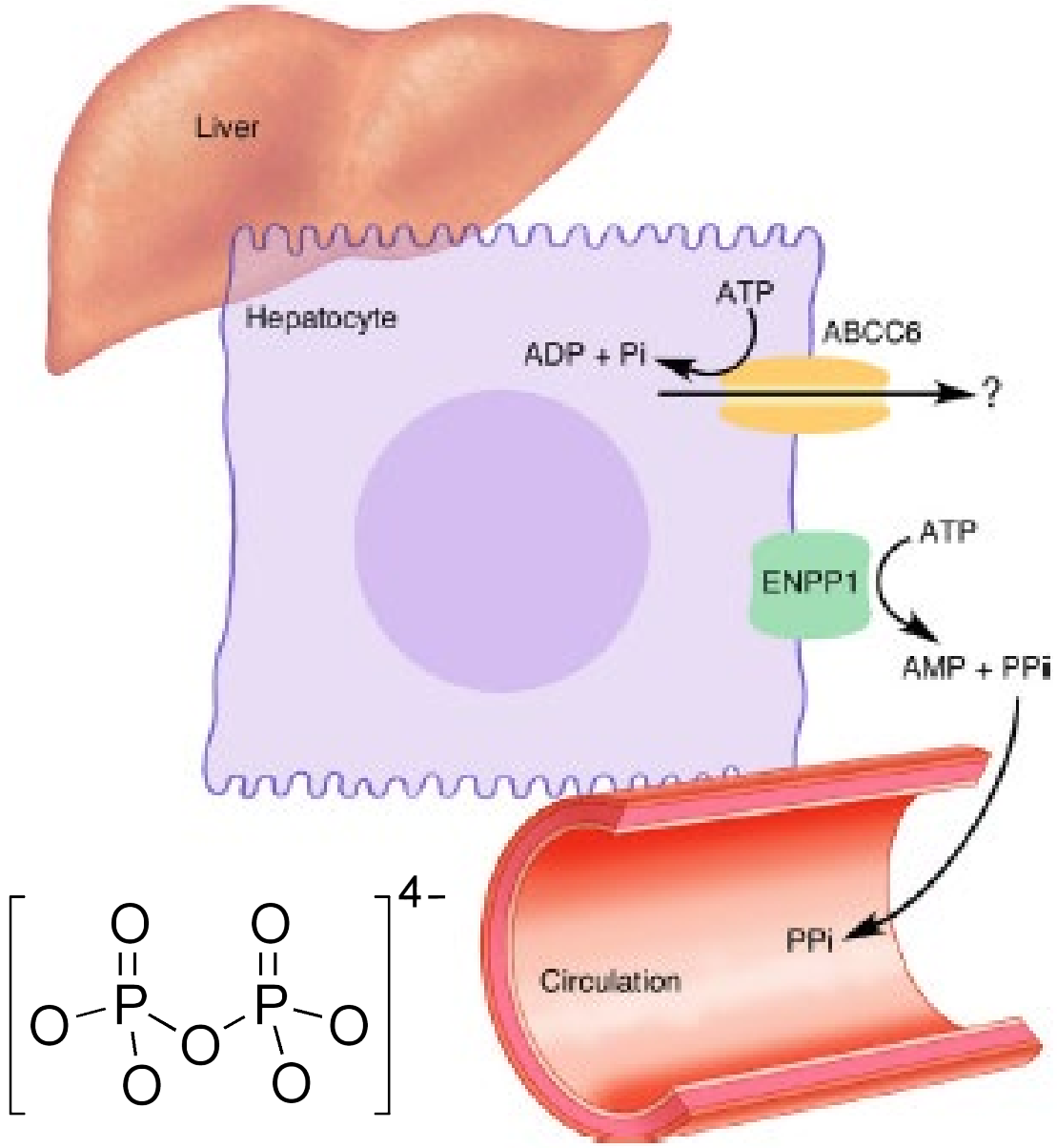
*(*** p < 0.001 ;**** p < 0.0001)*

Preliminary data

Natural history and emerging therapies for ENPP1 deficiency

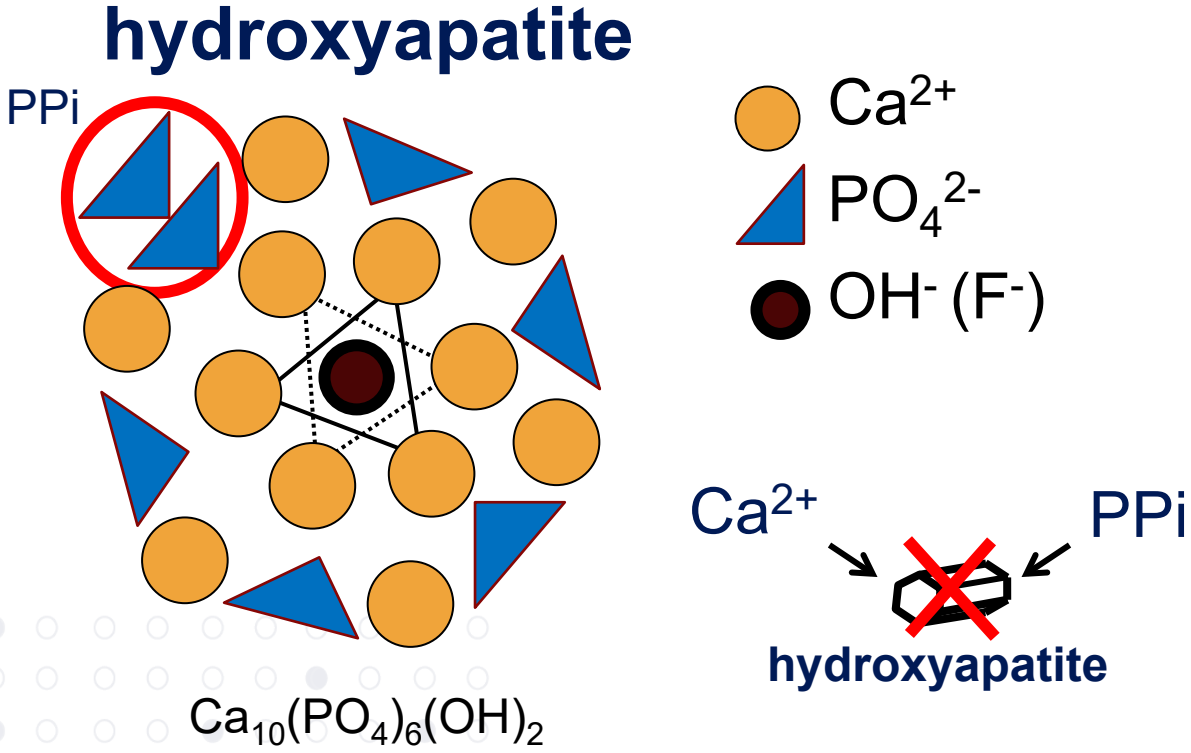
ENPP1 function

- Systemic:
  - main source of circulating PPi
- Local:
  - main source of extracellular PPi (e.g., VSMCs)

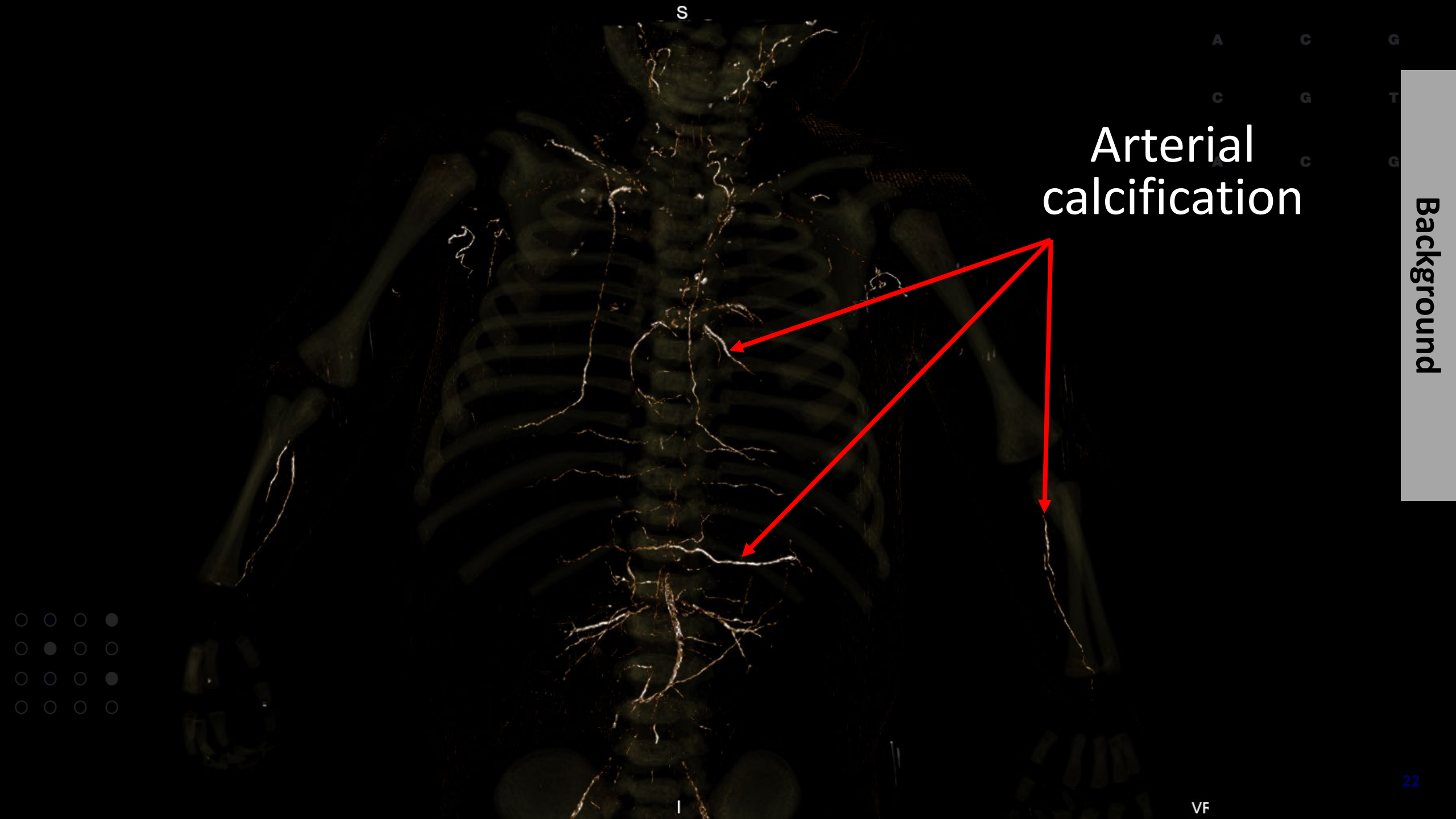


Ziegler, Gahl & Ferreira. *Genetics of Bone Biology and Skeletal Disease*; 2018

Role of PPi in mineralization

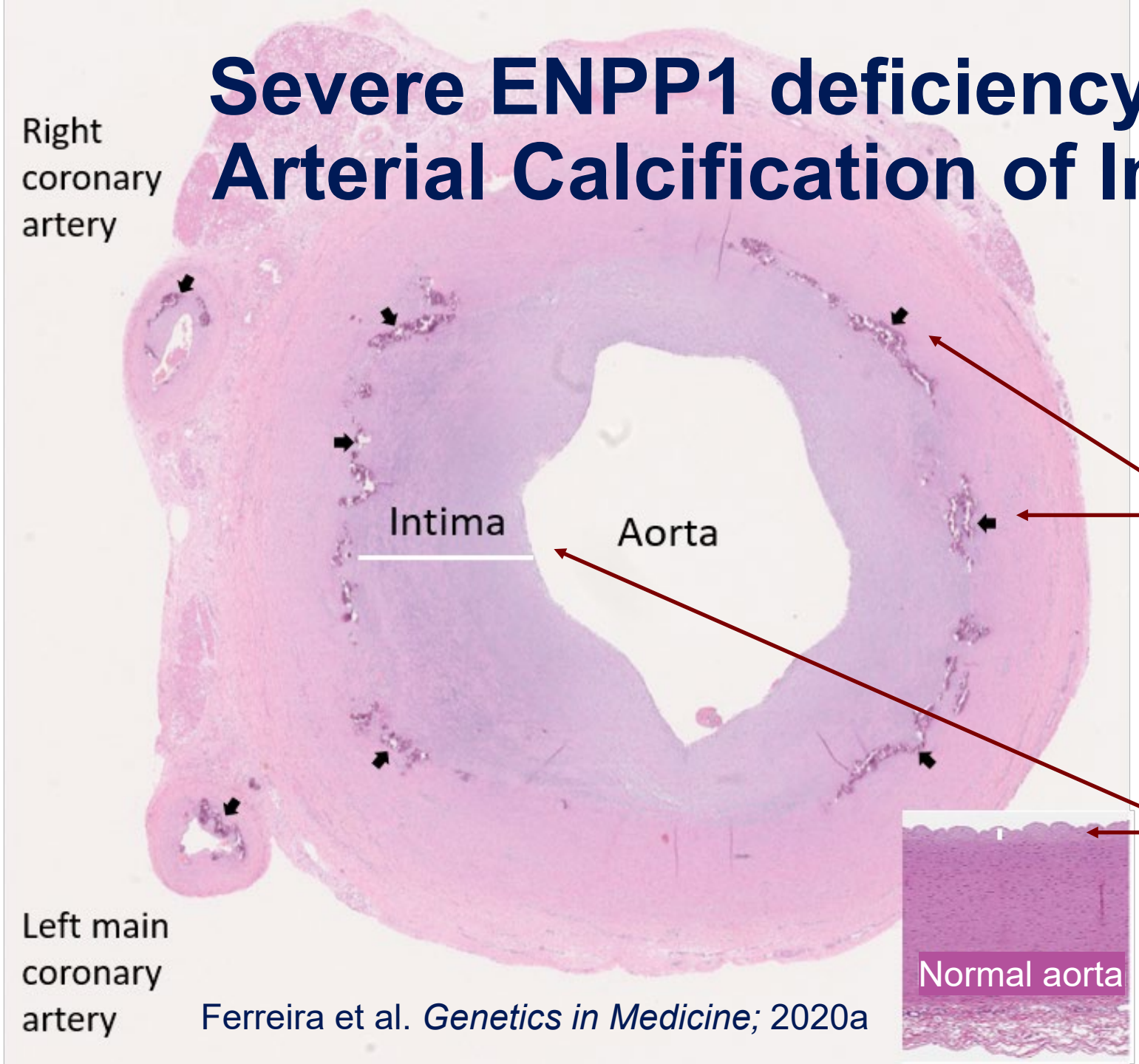


- Surface adsorption of PPi disrupts crystal structure
- Inhibits mineralization at [ ] as low as 10^{-7} M
  - plasma [ ] ~10^{-5} M



Arterial calcification

Severe ENPP1 deficiency: Generalized Arterial Calcification of Infancy (GACI)

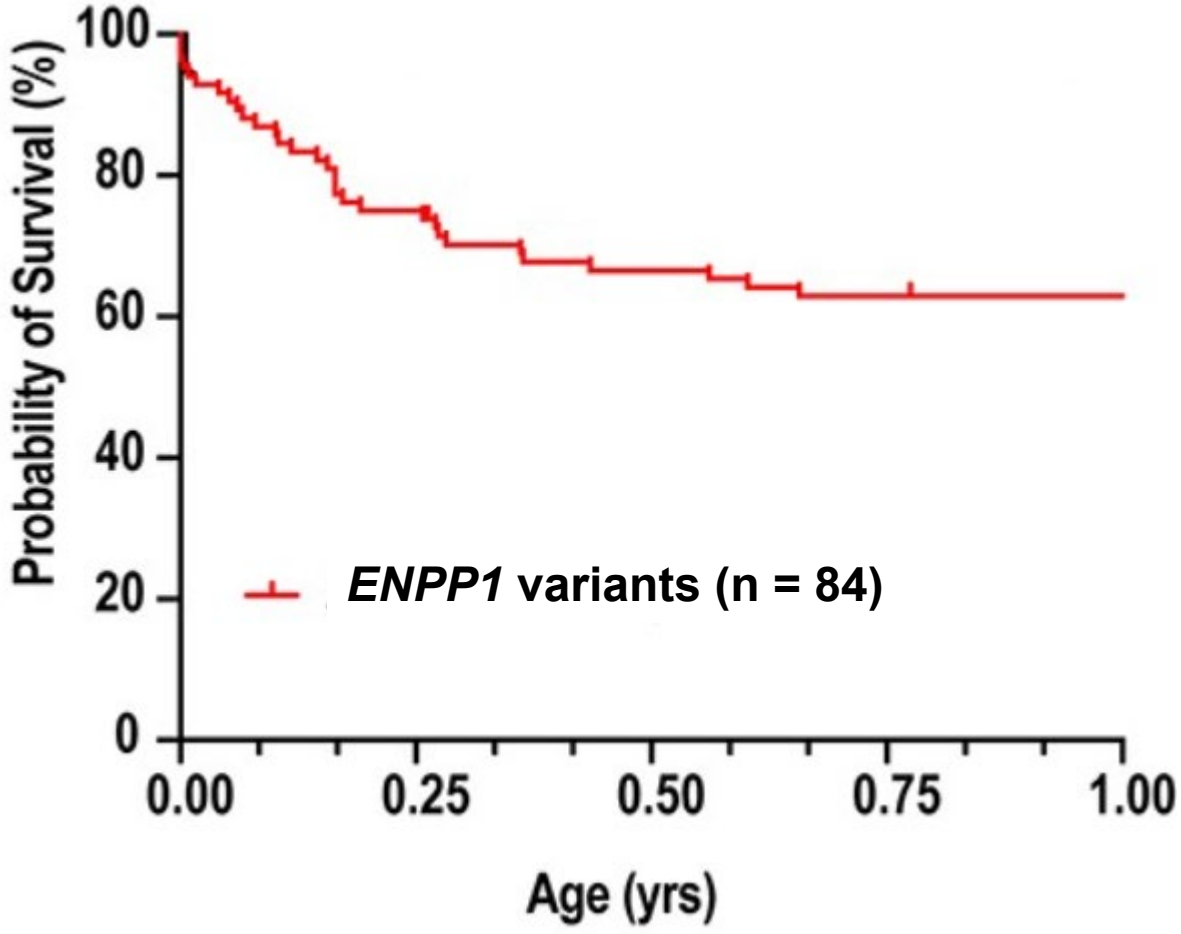


- Calcification along internal elastic lamina (arrowheads)
- Intimal proliferation (white line)

Ferreira et al. *Genetics in Medicine;* 2020a

High mortality

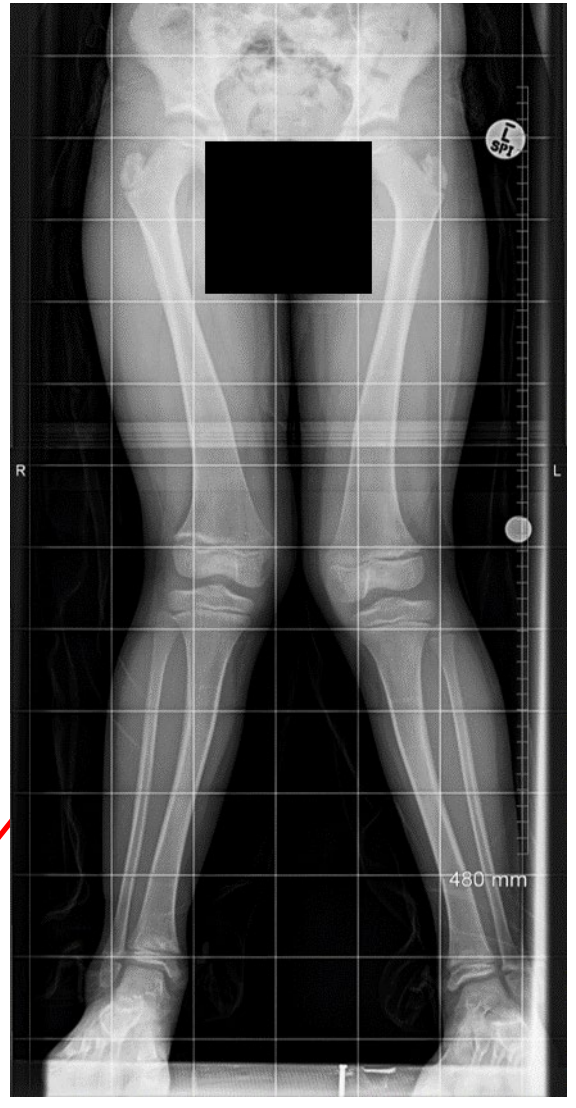
- Overall mortality: 40.5%
  - 4.7% in utero or stillborn
  - 36.8% before 6 months (critical period)



Ferreira et al. *Journal of Bone and Mineral Research*; 2021a

ENPP1 deficiency also causes FGF23-mediated hypophosphatemic rickets

- GACI survivors or patients with milder forms of ENPP1 deficiency → Autosomal Recessive Hypophosphatemic Rickets type 2 (ARHR2)
- Mediated by FGF23
- Cause of FGF23 increase unknown



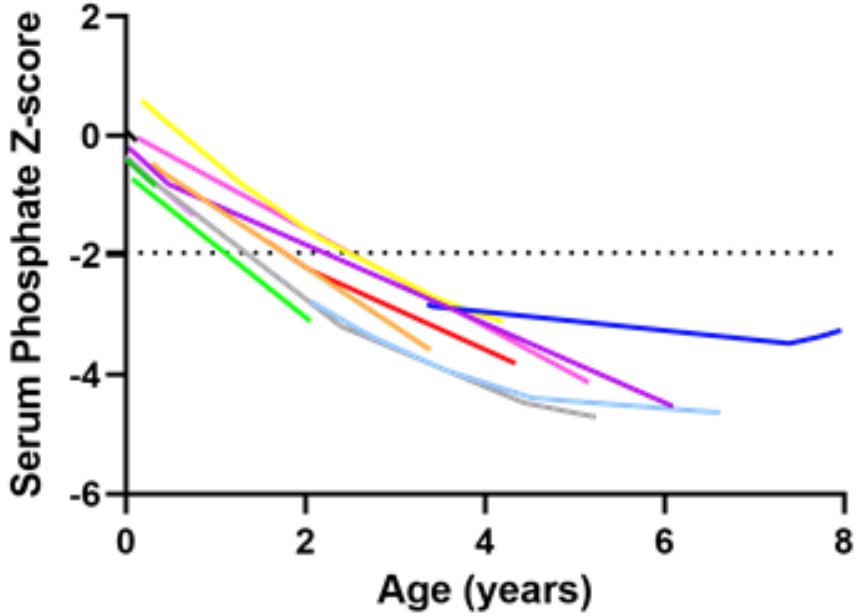
Genu valgum (knock knees)

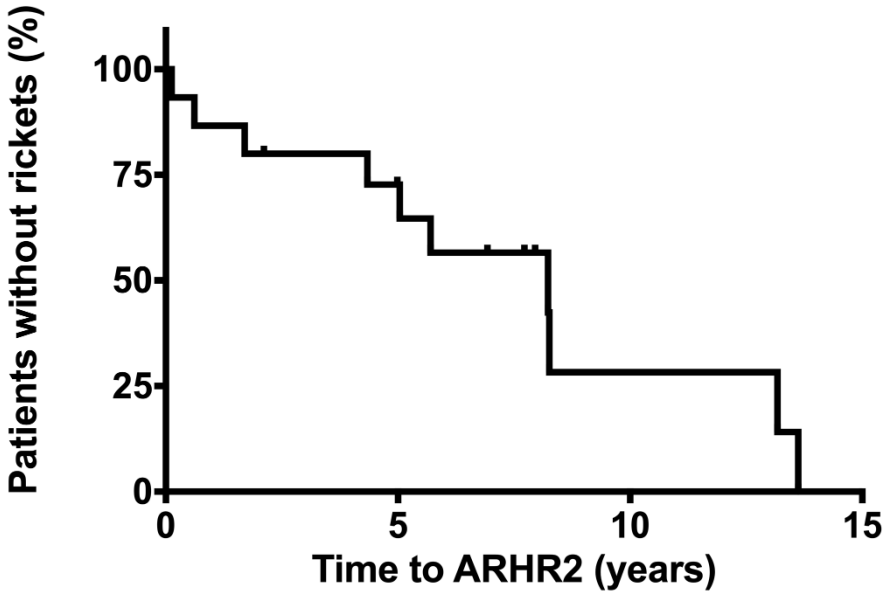
Hypophosphatemic rickets

- Average age of onset of hypophosphatemia: 1.6 yr

- Probability of developing rickets:
  - 20% by 2 yo
  - vast majority by adolescence

Ferreira et al. *Genetics in Medicine;* 2020a

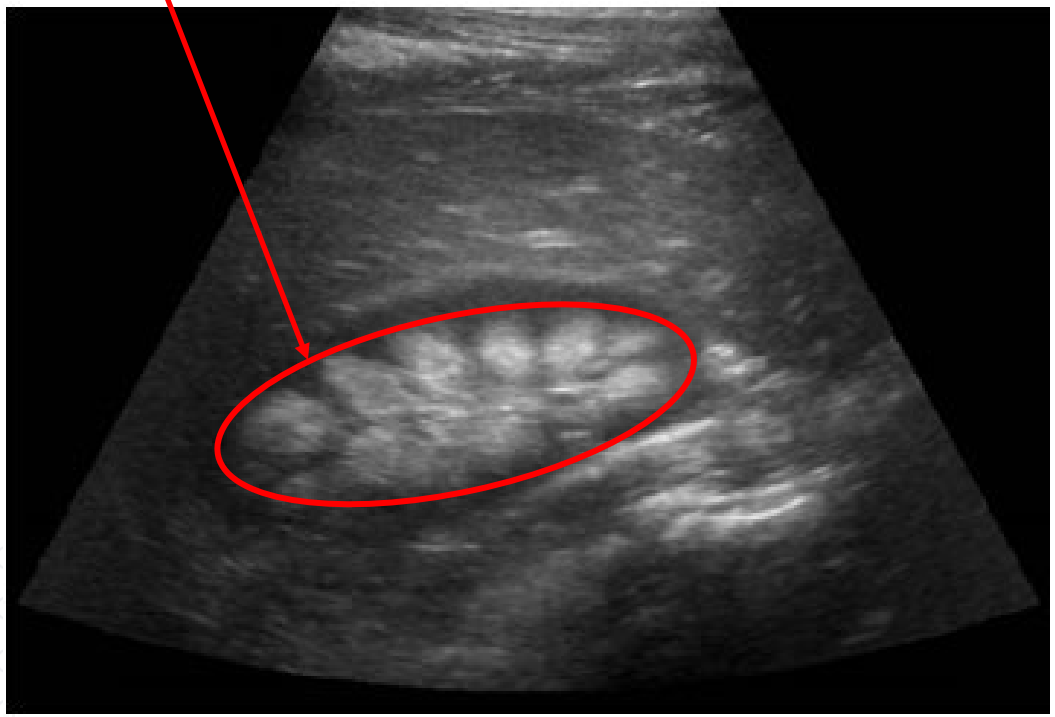




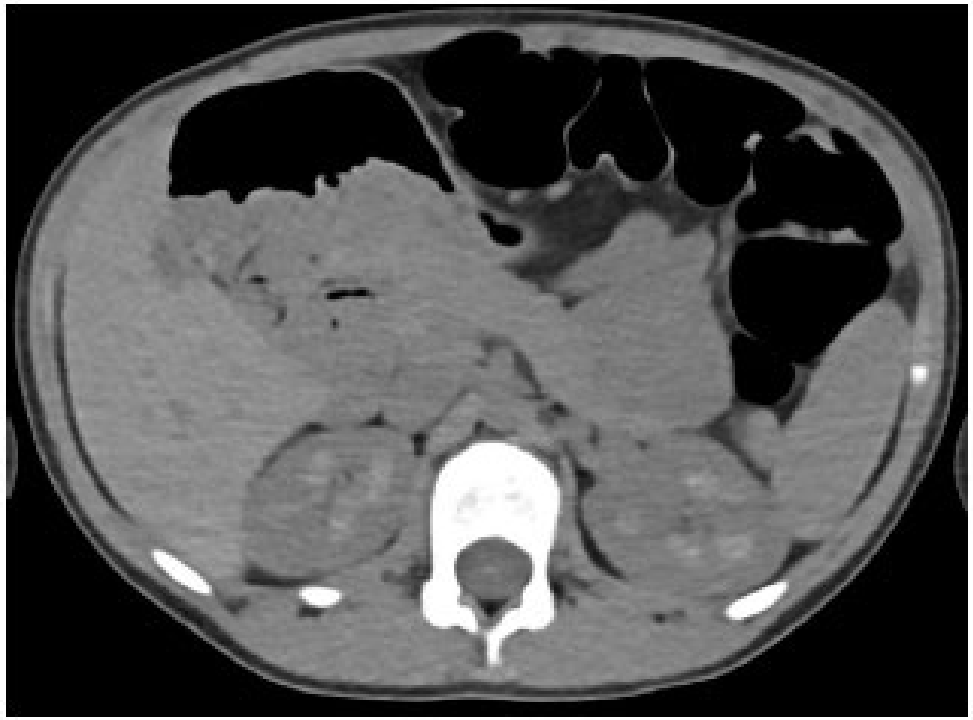
Preliminary data

Standard rickets treatment leads to kidney calcification

Medullary nephrocalcinosis (ultrasound, 9.7 years)



Bilateral calcification of renal pyramids (CT, 8.3 years)

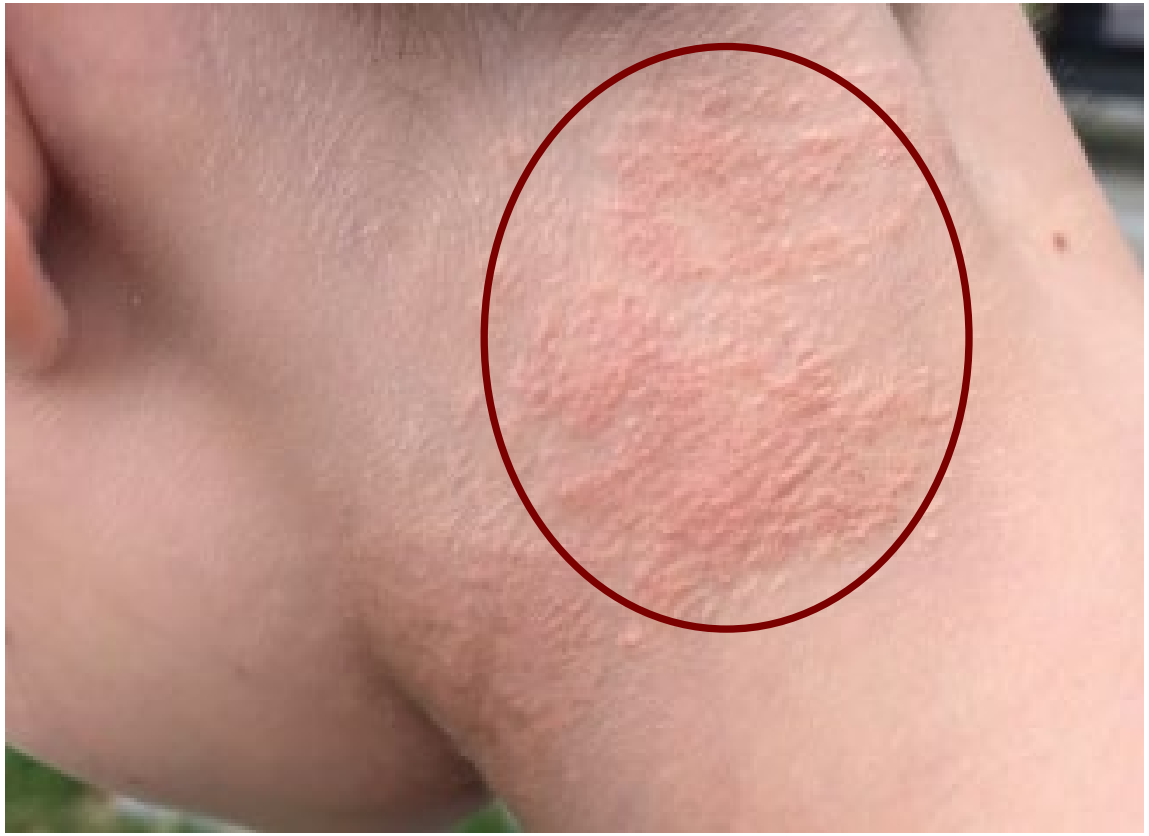


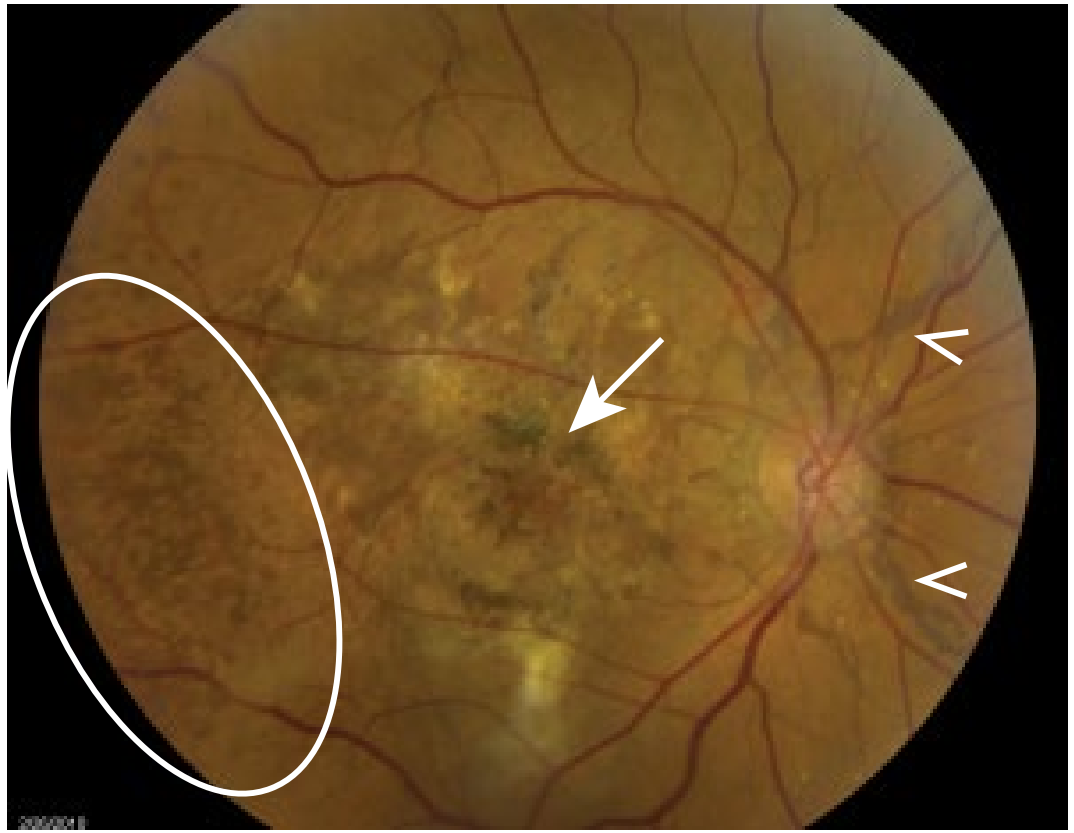
Nephrocalcinosis: 5/10 patients receiving conventional therapy, 0/7 patients not receiving therapy

Ferreira et al. *Journal of Bone & Mineral Research*, 2021b

Preliminary data

Other manifestations: Pseudoxanthoma elasticum (PXE)





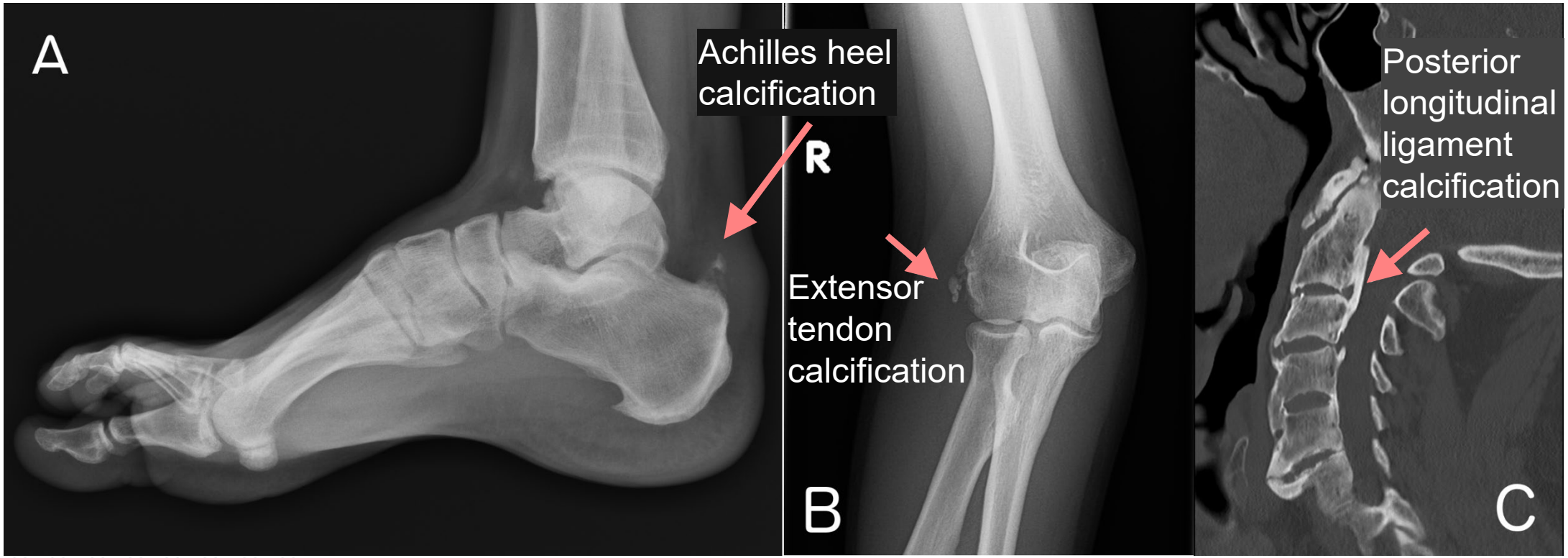
Ferreira et al. *Genetics in Medicine;* 2020a

Circle: peau d'orange
Carets: angioid streaks
Arrow: retinal bleeding → scarring

Preliminary data

Skeletal complications: Enthesopathy

- Major morbidity in adults related to enthesis calcification



Ferreira et al. *Genetics in Medicine*, 2020a

Preliminary data

Musculoskeletal symptoms

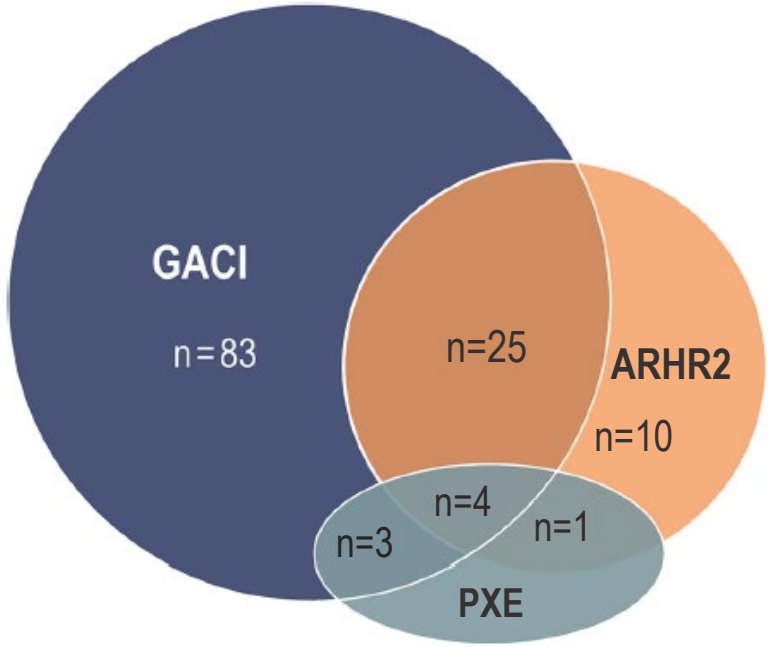
- Brief Pain Inventory – Short Form
  - 6/7 patients (mean age 31.6 yo) had musculoskeletal pain
  - 5 required treatment but none achieved complete pain relief (relief percentage: 20-70%)

- PROMIS Physical Function form
  - 1/7: mild impairment
  - 4/7: moderate impairment
  - 1/7: severe impairment

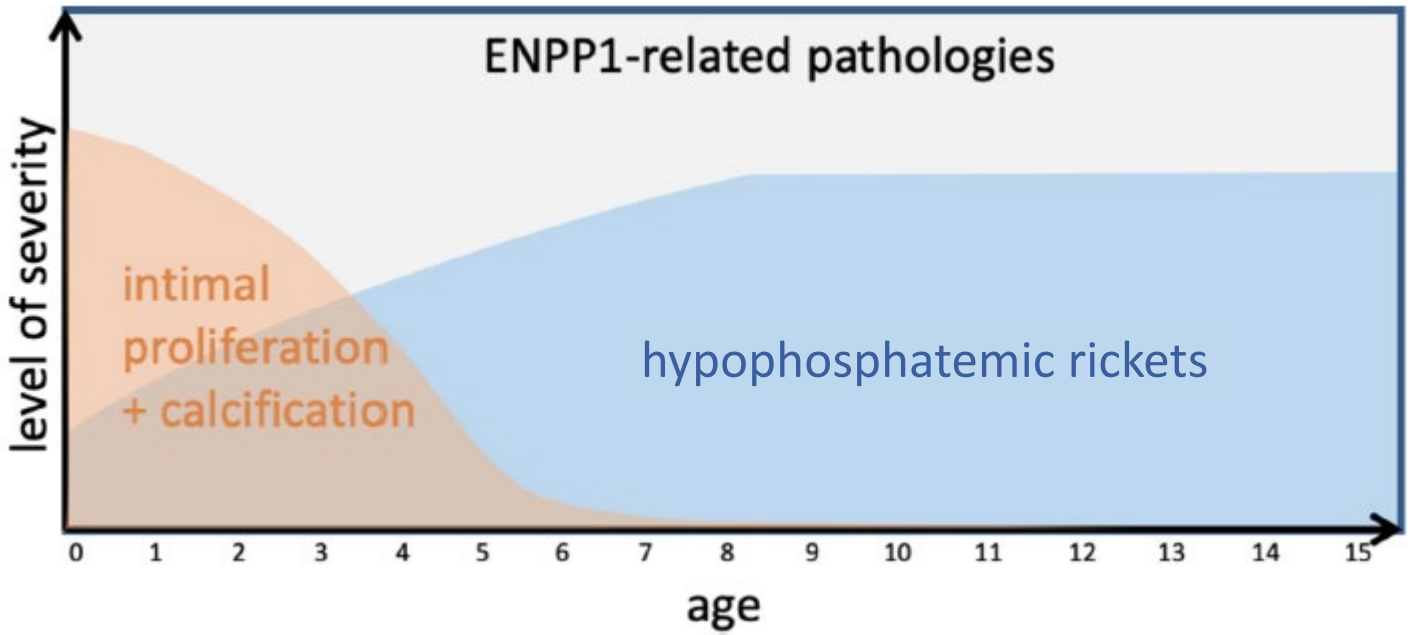
Ferreira et al. *Journal of Bone and Mineral Research*; 2021c

Preliminary data

ENPP1 deficiency: Multiple phenotypes

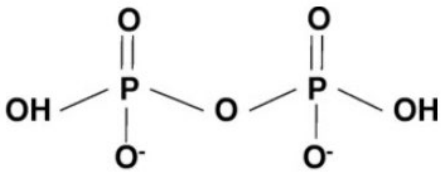


Ferreira et al. *Journal of Bone and Mineral Research*; 2021a

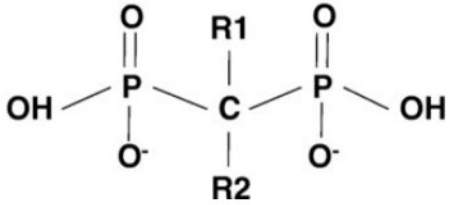


Höppner et al. *Bone;* 2021

Therapy - Bisphosphonates



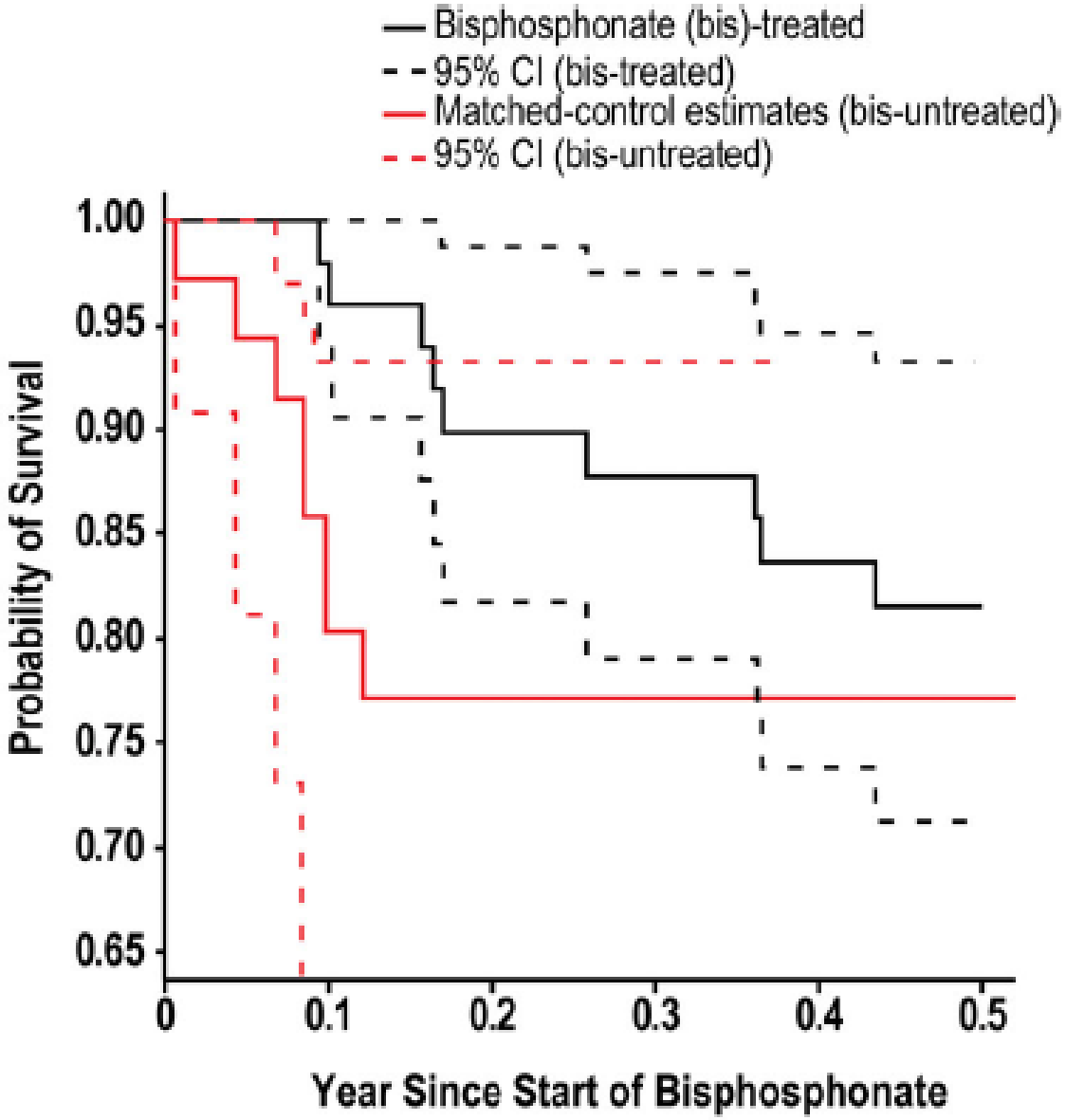
Pyrophosphate



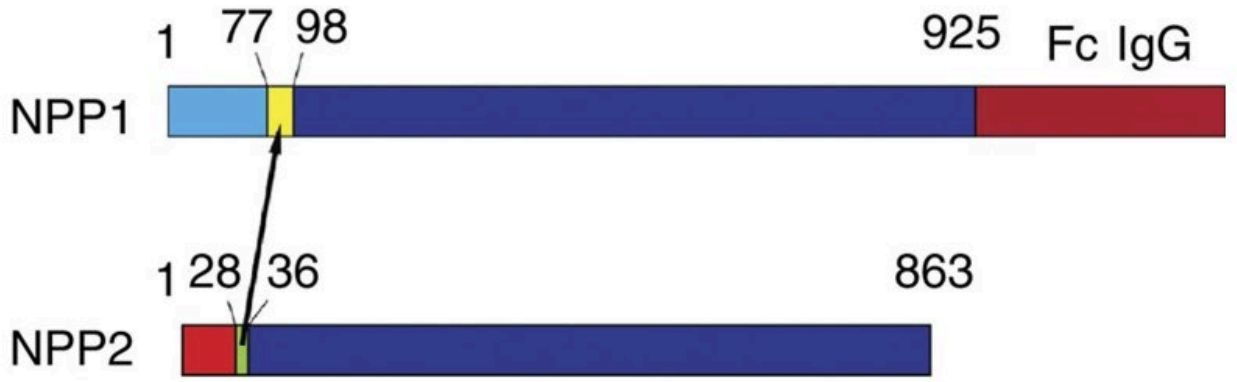
Bisphosphonate

- Trend towards benefit but no statistical significance

Ferreira et al. *Journal of Bone and Mineral Research*; 2021a



Enzyme replacement therapy (ERT) for ENPP1 deficiency



Preliminary data

ARTICLE

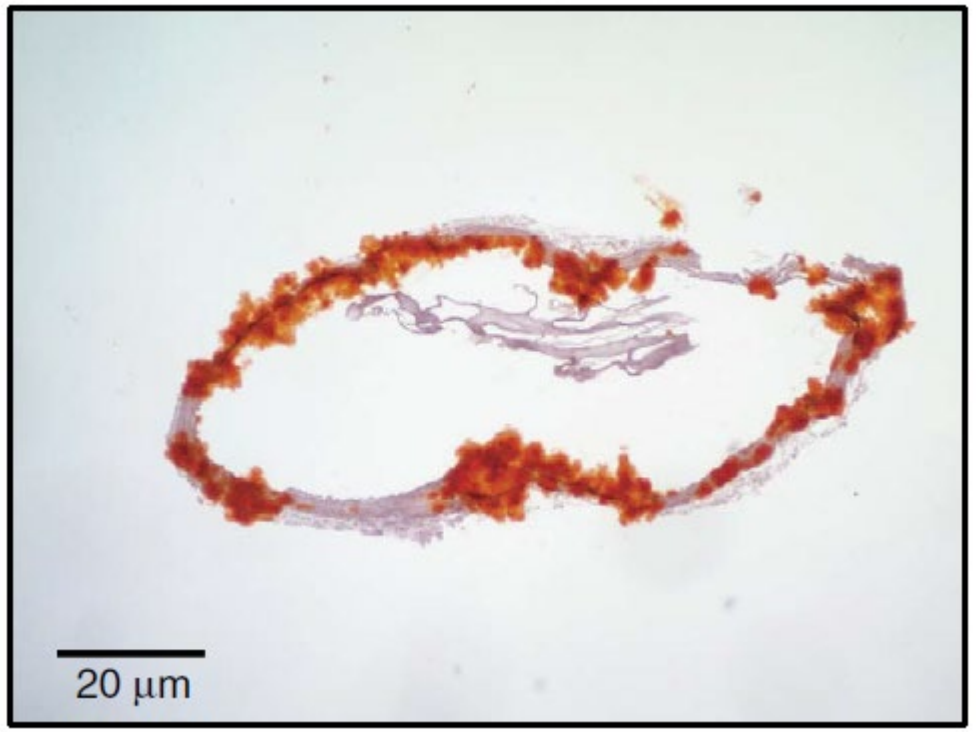
Received 11 May 2015 | Accepted 23 Oct 2015 | Published 1 Dec 2015

DOI: 10.1038/ncomms10006 OPEN

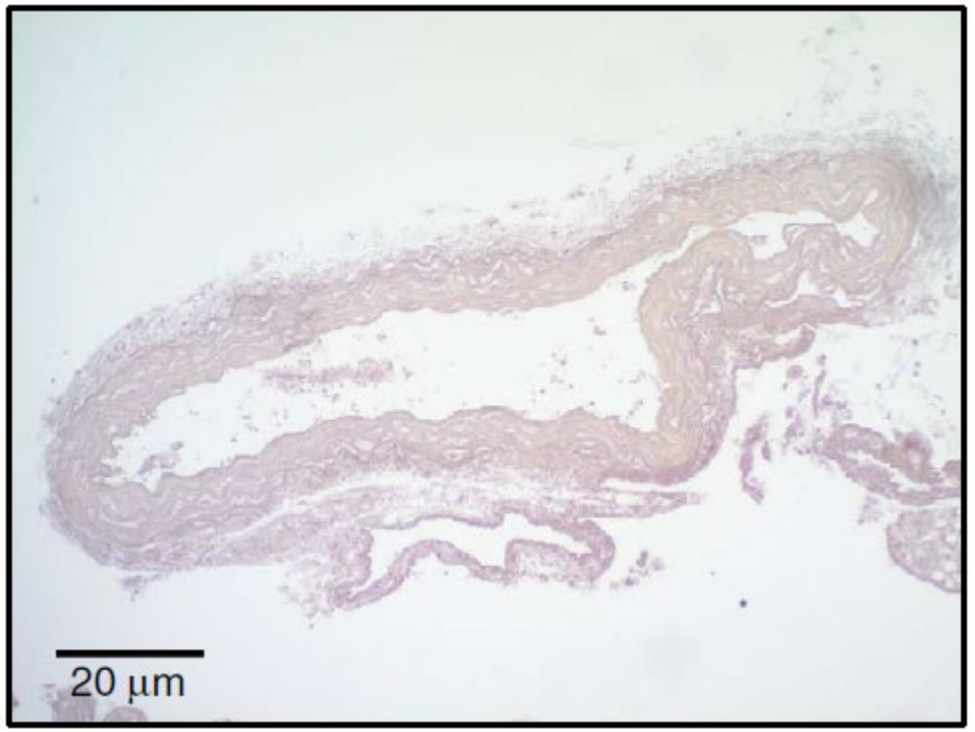
ENPP1-Fc prevents mortality and vascular calcifications in rodent model of generalized arterial calcification of infancy

ERT prevents vascular calcification in Enpp1-deficient mice

Untreated Enpp1$^{asj/asj}$



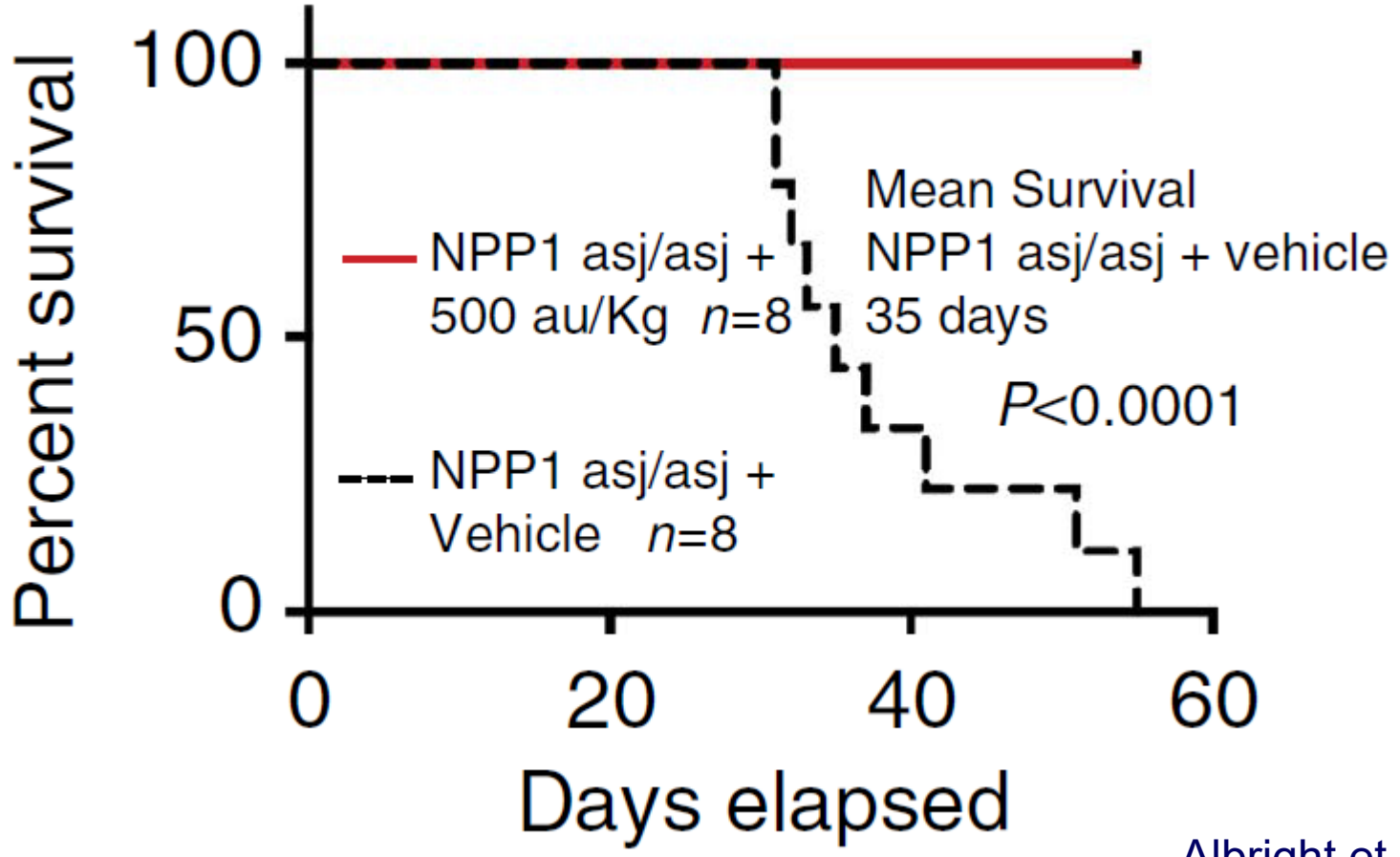
Treated Enpp1$^{asj/asj}$



Albright et al. *Nature Communications*, 2015

Preliminary data

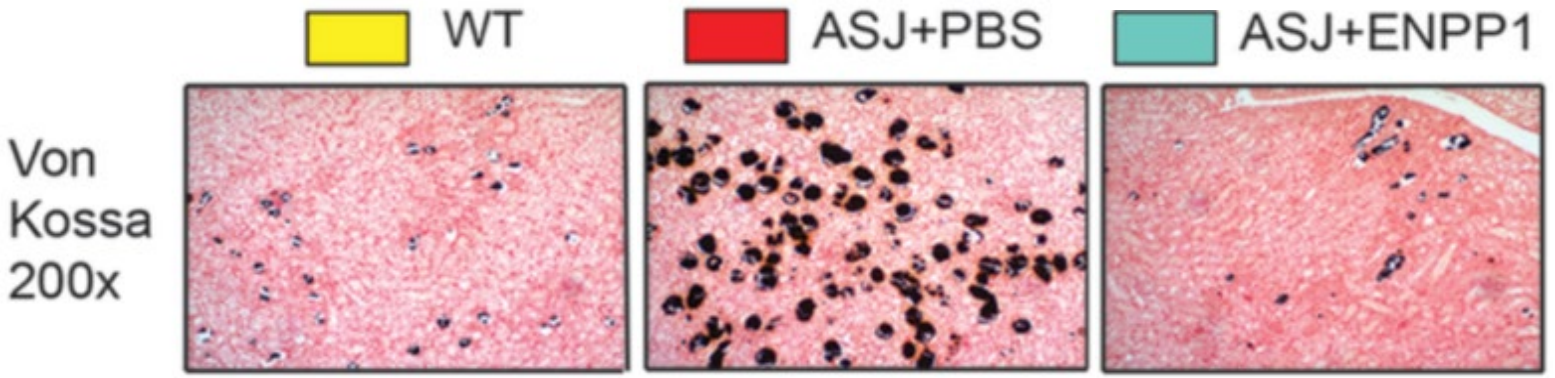
ERT prevents mortality in Enpp1-deficient mice

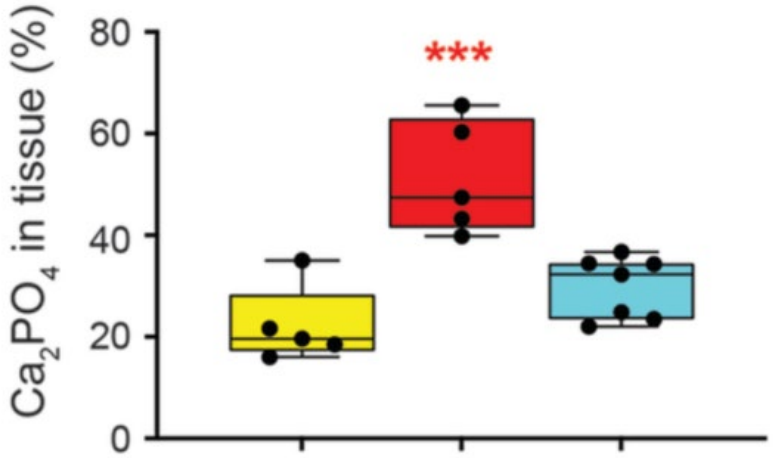


Albright et al. *Nature Communications*, 2015

Preliminary data

ERT prevents nephrocalcinosis





- No statistical significance between WT and *Enpp1*$^{asj/asj}$ mice treated with Enpp1-Fc
- Untreated *Enpp1*$^{asj/asj}$ mice experienced about 2-fold increase in nephrocalcinosis (*** $p<0.001$)

Ferreira et al. *Journal of Bone and Mineral Research*; 2021b

Preliminary data

ERT increases bone mass

WT

Enpp1$^{asj/asj}$ + vehicle

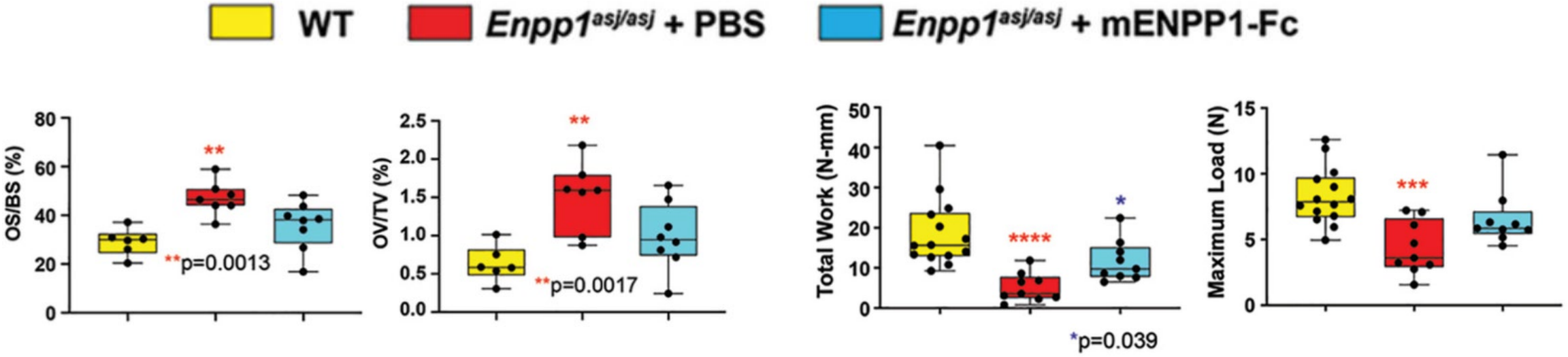
Enpp1$^{asj/asj}$ + Enpp1-Fc

Ferreira et al. *Journal of Bone and Mineral Research*; 2021b

Preliminary data

ERT improves skeletal mineralization and bone strength

- Histomorphometry (tibiae)
- 3-point bending (femurs)



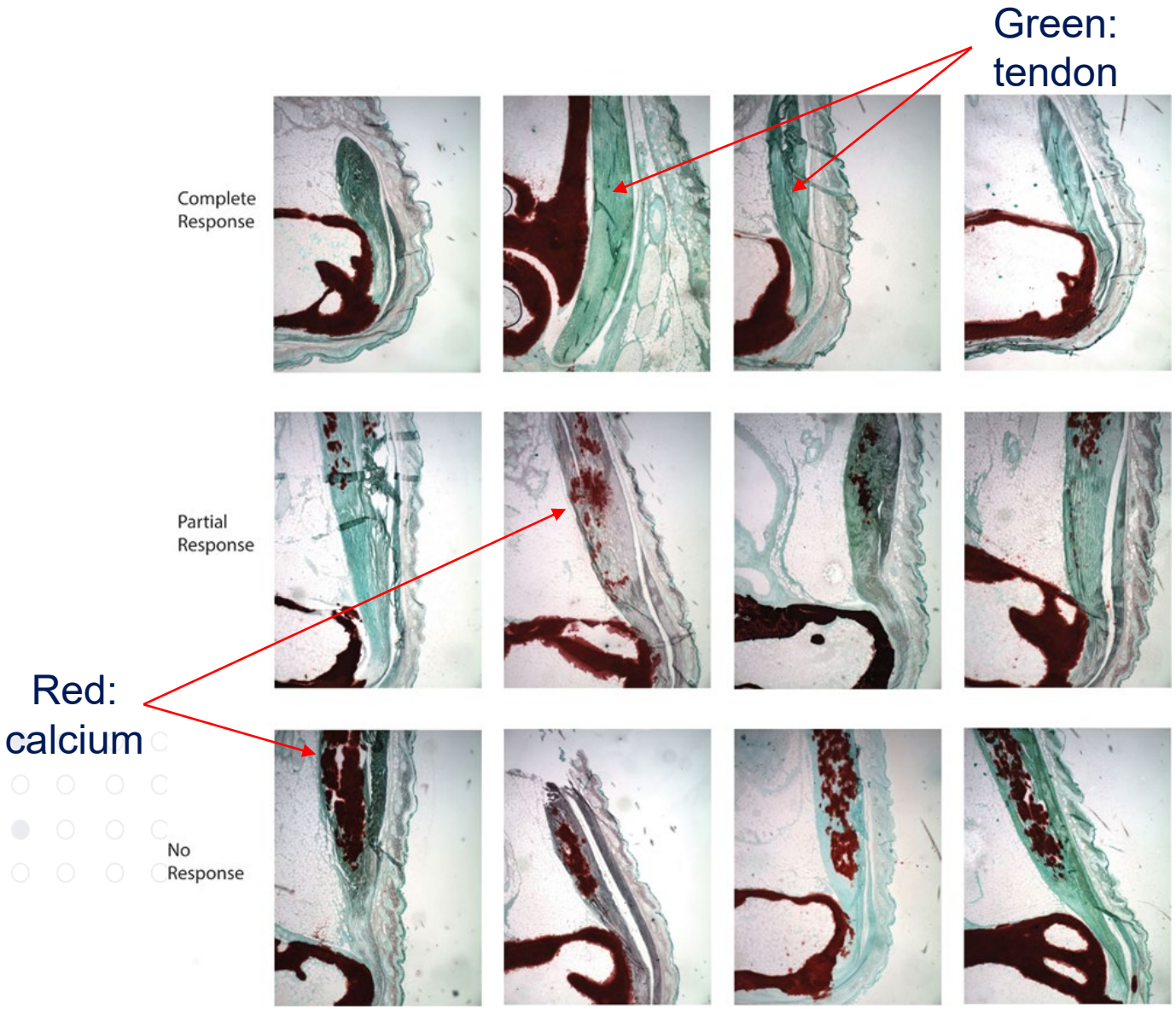
OS/BS: osteoid surface OV/TV: osteoid volume

*$p < .05$, **$p < .01$, ***$p < .001$, ****$p < .0001$ ANOVA comparison of means

Ferreira et al. *Journal of Bone & Mineral Research*, 2021b

Preliminary data

ERT partially prevents enthesopathies



Ferreira et al. *Journal of Bone & Mineral Research*, 2021c

Preliminary data

Clinical trials of ERT

https://www.inozyme.com/scientific-focus/clinical-trials/

Evaluation of Safety, Tolerability, and Efficacy of INZ-701 in Adults With ENPP1 Deficiency

The purpose of this study is to assess the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of multiple ascending doses of INZ-701, an ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) enzyme replacement therapy for the treatment of ENPP1 Deficiency. The goal of the study is to identify a dose regime for further clinical development in the treatment of ENPP1 Deficiency.

STATUS:
ACTIVE< ENROLLMENT COMPLETE

PHASE:
PHASE 1/2

AGE:
18 YEARS TO 64 YEARS

The ENERGY 3 Study: Evaluation of Efficacy and Safety of INZ-701 in Children With ENPP1 Deficiency

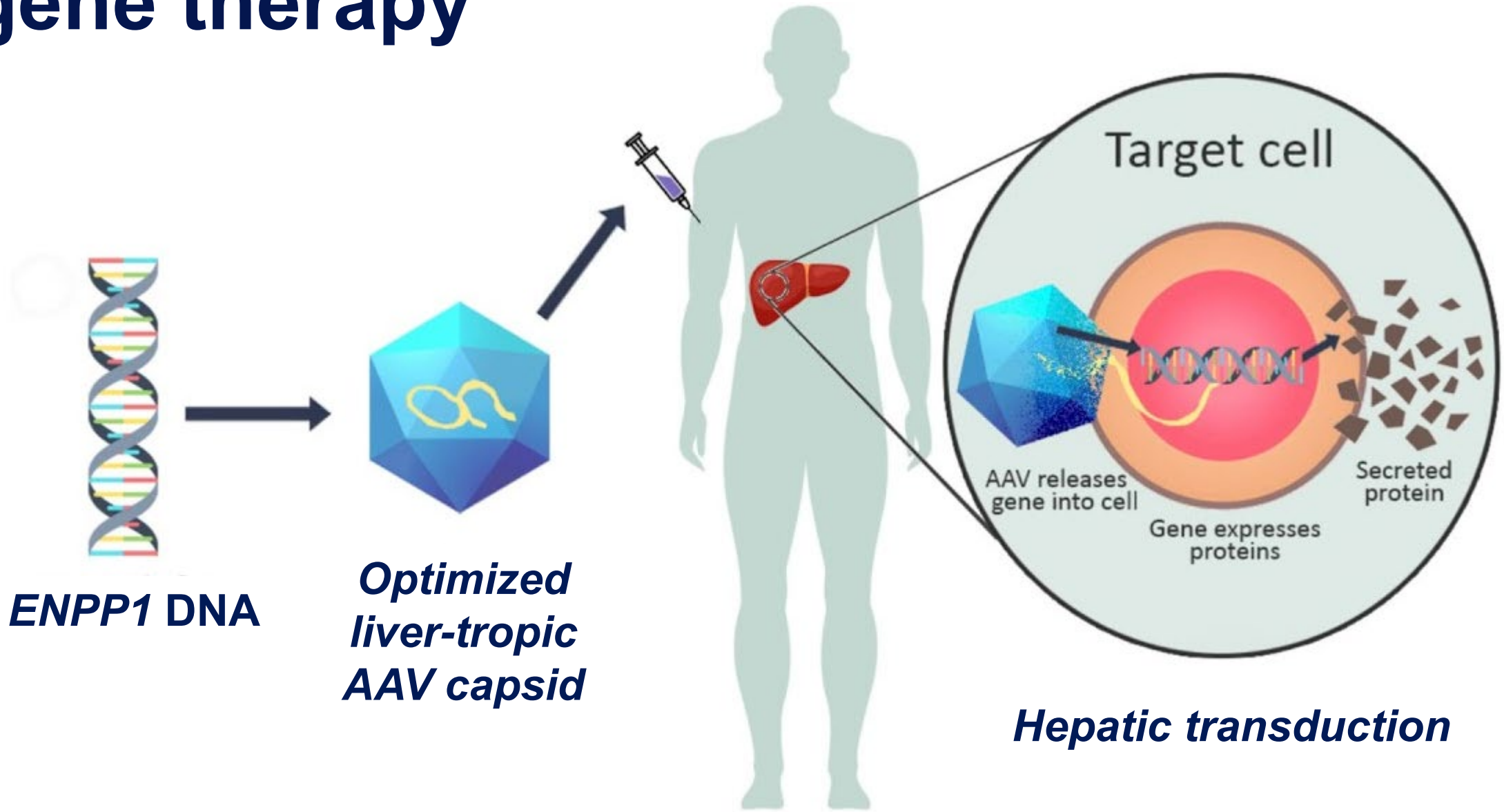
The primary purpose of Study INZ701-106 (The ENERGY 3 Study) is to assess the efficacy and safety of INZ-701 in children with ENPP1 Deficiency.

STATUS:
RECRUITING

PHASE:
PHASE 3

AGE:
1 YEAR TO 12 YEARS

Future directions: From soluble ERT to gene therapy



***ENPP1* DNA**

Optimized liver-tropic AAV capsid

Hepatic transduction

Concluding remarks